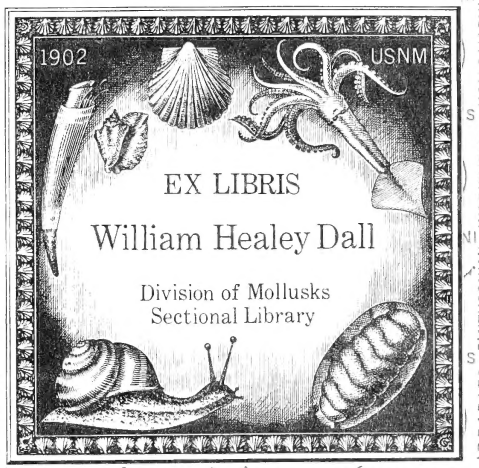

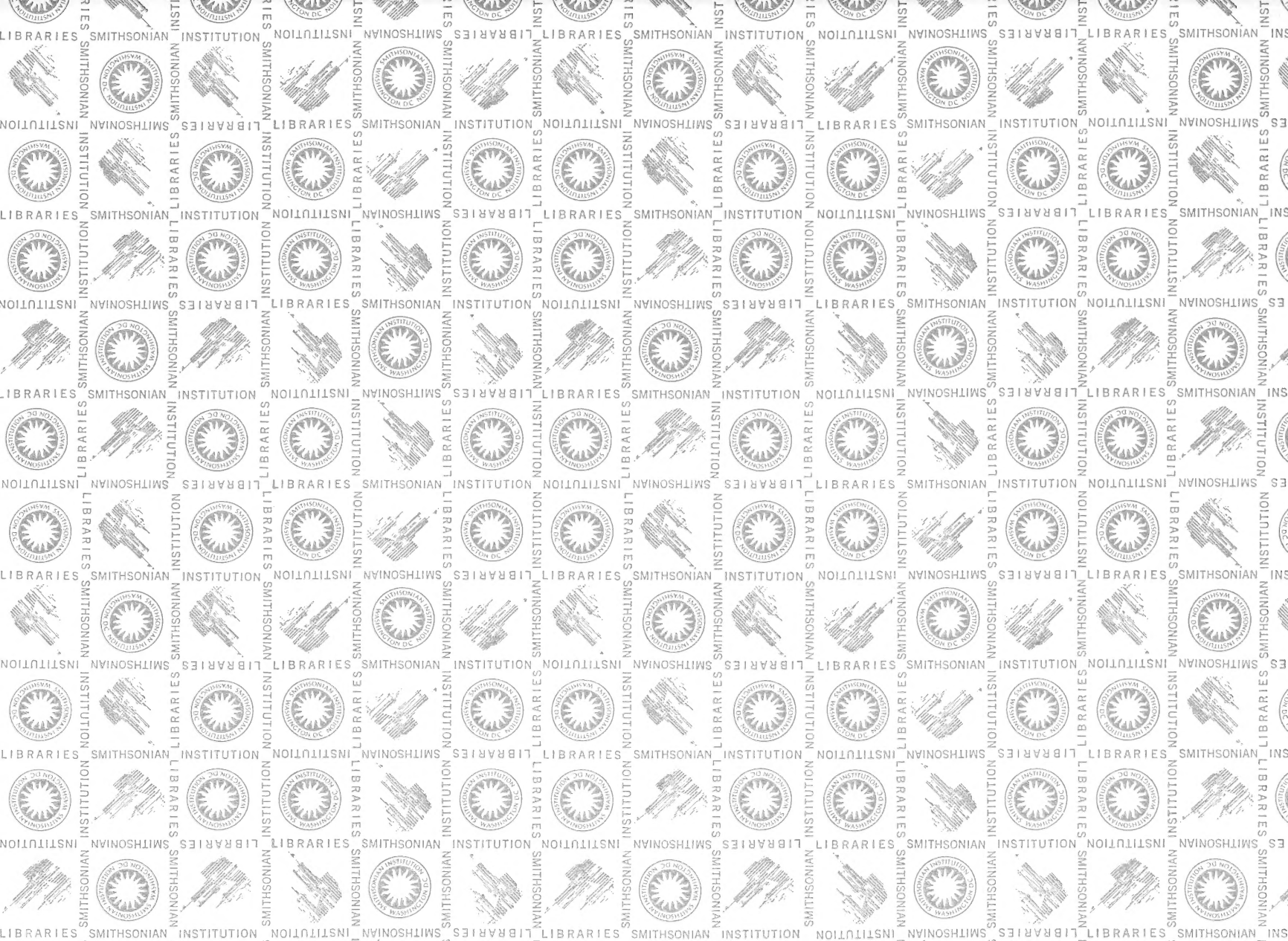
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KARYOKINESIS AND CYTOKINESIS

IN THE

Maturation, Fertilization and Cleavage

OF

CREPIDULA and other GASTEROPODA

BY

EDWIN G. CONKLIN,
PROFESSOR OF ZOOLOGY.

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PART I. KARYOKINESIS.

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PART II. CYTOKINESIS.

CONTENTS.

KARYOKINESIS AND CYTOKINESIS IN THE MATURATION, FERTILIZATION AND CLEAVAGE OF CREPIDULA AND OTHER GASTEROPODA.[1]

By Edwin G. Conklin, Ph.D.

I.

Introduction.

Cell division, in a broad sense, includes not only nuclear division and the separation of daughter cells, but also all the phenomena which lead up to these processes and which follow them; the terms *Karyokinesis* (Schleicher '78) and *Cytokinesis* (Whitman '87) are used in this paper to include these nuclear and cytoplasmic activities of the entire cell-cycle from one division to the next. Flemming ('82) has objected to the term *Karyokinesis* on the ground, among others, that nuclear movements are not characteristic of indirect division. But in view of the extensive movements of both nuclei and cytoplasm, which occur in the cell divisions described in this paper, the terms *Karyokinesis* and *Cytokinesis* have peculiar appropriateness.[2]

The phenomena of cell division still include an extraordinary number of dark problems, in spite of the fact that "all the search-lights of the biological sciences have been turned upon the cell." Confusion and contradiction exist as to the nature and metamorphoses of the centrosome and central spindle, the origin and fate of the amphiaster, the characteristics and history of the attraction sphere, the existence or non-existence of a specific substance (Archoplasm, Kinoplasm, etc.) whose primary function is the division of the cell. Still less complete is our knowledge of the interrelation of nucleus and cytoplasm during the various phases of division, of the phenomena and significance of the movements of cells and cell constituents and of the chemical, physical and physiological principles involved in the division of nucleus and cell body.

In the early development of the egg, cell divisions have a peculiar interest because of their bearings on problems of heredity and differentiation. Here are found phenomena of the most general occurrence and of the deepest significance, viz.: the maturation, fertilization and cleavage of the egg and the early differentiation of the embyro. The bearings of the phenomena of maturation and fertilization upon

[1] From the Zoological Laboratory of the University of Pennsylvania.

[2] On the other hand Flemming's term *mitosis* commends itself because of its brevity, and it is frequently employed throughout this paper.

more densely staining body, usually eccentric in position, Plate I, fig. 1. The chromatin is in the form of small granules, varying in size and irregular in shape, which are attached to linin threads stretching through the vesicle. Many of these granules can be seen to be three- or four-parted, though others are rounded or irregular in shape. The four-parted granules are larger than the others but all are extremely small; their method of formation was not observed. At this stage no trace of centrosomes can be found anywhere in the egg.

2. Egg Laying.—After the eggs leave the ovary they descend to the lower enlarged part of the oviduct, where they meet spermatozoa from the *receptaculum seminis* and together with an albuminous fluid are surrounded by a glairy, mucous substance, which hardens into capsules. These capsules are attached together in a cluster and are fastened by a common stalk to the object upon which the female is seated. All the eggs laid by one individual begin development at nearly the same time and proceed with remarkable uniformity, so that whenever examined they are all found to be in approximately the same stage.

The earliest stages of free eggs which I have seen were taken from the oviduct while the capsule was being formed. The outline of the egg at such a stage is usually elliptical or irregular, being in marked contrast with the spherical form which it attains after the entrance of the spermatozoon. The germinal vesicle is slightly eccentric in position and immediately around it there is a small amount of cytoplasm in the interstices between the yolk spheres; elsewhere in the egg the yolk spheres are closely crowded together. The nucleolus is now a single, homogeneous body and frequently exhibits an alveolar or recticular structure. The chromatin granules are rounder and a little larger than in the ovarian egg and many of them are arranged in rows or strands, fig. 2. In one egg of this stage I observed two minute granules in the cytoplasm, close to each other and in contact with the nuclear membrane; these are possibly the centrosomes, though no polar radiations or central spindle was observed. At this stage the spermatozoon has not entered the egg (Plate I, fig. 2).

B. Maturation Divisions.

1. Nucleus.—The earliest trace of the first maturation division which I have seen appears about the time of the entrance of the spermatozoon. The centrosomes are now plainly visible, being surrounded by a few short radiations, and are connected by a central spindle. At the same time the nuclear membrane is indented opposite the poles of this spindle and fibres can he traced from the centrosomes to these indented areas, Plate I, figs. 3 and 4.

At this stage the germinal vesicle contains a great number of chromatin granules which are connected together by linin threads, also a single extremely large nucleolus, while the nuclear sap fills all the interstices within the nucleus and constitutes the greater part of its bulk.

(a). *Chromatin.*—A few of the chromatin granules are larger than the others and their form is spherical, 2-lobed, 3-lobed or 4-lobed. They are probably identical with similar granules found in the pre-division stages. They differ much in

size and for this reason their number cannot readily be determined since they grade down to the smaller granules, which are innumerable (Plate I, figs. 3 and 4). These larger granules continually increase in size and become the chromosomes of the first maturation spindle; some of these granules stain less deeply at the center than at the periphery. As the chromosomes grow in size the remaining chromatin granules, which constitute the chief bulk of chromatin within the germinal vesicle, grow smaller and smaller and are gradually dissolved; on the disappearance of the nuclear membrane they escape with the nuclear sap into the cytoplasm, figs. 5, 6 and 7. At the same time the linin threads, which were plainly visible at an earlier stage, fig. 4, are no longer to be seen, but the arrangement of some of the granules in radiating lines, fig. 5, is probably to be taken as evidence that some of these threads still exist. In the early stages of the first maturation division, all the chromatin granules stain alike, in later stages the chromosomes stain more densely with nuclear stains, while the remaining granules show an increasing affinity for plasma stains. In such stages as are shown in figs. 3 and 4, there are no perceptible differences, save size only, between the granules which become chromosomes and those which dissolve; the fact, however, that the history of these two groups is so different shows there is some fundamental difference between them. It is highly probable that the faintly staining granules which are ultimately dissolved or transformed into linin are identical with the lanthanin or oxychromatin of Heidenhain ('94).[1]

(b). *Nuclear Sap.* Before the solution of the oxychromatin granules and nucleolus begins, the nuclear sap is a clear and almost non-colorable fluid. As the solution of these elements progresses the nuclear sap becomes granular and tingible, staining blue or purple with Delafield's hæmatoxylin alone, though it stains deeply with plasma stains, such as eosin or orange G. when these are used after the hæmatoxylin. Even after the nuclear membrane has entirely disappeared the nuclear sap and oxychromatin can still be recognized as a granular mass, figs. 5, 6, 7.

Korschelt ('95) has observed a similar increase in the staining properties of the nuclear sap of *Ophryotrocha*, where the "Kernsaft" stains more and more deeply as division advances until it becomes so dark that the chromatin threads are invisible. Then the sap loses some of its staining qualities and, at the same time, dissolved nucleolar substance is probably added to the chromatin threads.

[1] The term *Achromatin*, as used and defined by Flemming ('82, p. 375), is limited to "that formed substratum of nuclear structures, as well as of the division figures, which is not colored by nuclear stains." As thus defined, it is applicable only to the linin, and is not even applicable to it at all stages in the cell cycle, since the linin also is colored by nuclear stains at certain stages. Furthermore, it is extremely probable that oxychromatin is transformed into linin at certain stages, and that oxychromatin and perhaps linin are sometimes dissolved in the nuclear sap. This interrelation of these various parts of the nuclear substance makes it impossible to apply the terms "chromatin" and "achromatin" as used by Flemming. Nevertheless, it is convenient to have a term which will include all of the nuclear constituents which do not form chromosomes, as contrasted with that which does. Since the term achromatin has frequently been used in this sense, and since I am unwilling to further cumber cytological nomenclature with new names, I shall employ the term "achromatin," or "achromatic substance of the nucleus" to include all the contents of the nucleus except the chromatin, and even that portion of the chromatin which does not form chromosomes. As thus used it includes linin, oxychromatin, nuclear membrane and nuclear sap.

(c). *Nucleolus*.—The chromosomes, which are at first widely scattered through the nucleus, figs. 3 and 4, gather together more closely and often lie immediately around and upon the nucleolus, figs. 5 and 6. In some cases it looks as if these chromosomes were being formed out of the substance of the nucleolus, and the fact that the nucleolus diminishes in size as the chromosomes increase lends color to this view. On the other hand, when the chromosomes first appear they are scattered through the entire nucleus and do not lie close to the nucleolus, and though it is possible that they may later receive substance from the dissolving nucleolus, it is impossible to suppose that they are fragments of the nucleolus. The latter is gradually dissolved without any fragmentation. Before the complete disappearance of the nuclear membrane the nucleolus has greatly diminished in size, and at the same time the nuclear sap stains more deeply, fig. 5. After the disappearance of the nuclear membrane the nucleolus comes to lie outside the spindle, while most of the chromosomes are found within it, though some of them may still be scattered among the polar radiations, fig. 7.

Within the cytoplasm the nucleolus continues to diminish rapidly in size and soon entirely disappears, figs. 8, 9, 12*a*. In this respect the history of the nucleolus in the first maturation of *Crepidula* is the same as has been described by Häcker ('93), Foot ('94), Mead ('95), Wheeler ('95), Obst ('99), and others, in a considerable number of animals.

(d). *Chromosomes*.—The shapes of the chromosomes of the first maturation spindle are shown in figs. 7–15 and in text fig. I. In the early prophase the most common form is that of a 3- or 4-lobed body; in fact, such bodies are found in the nucleus of the ovarian egg. There can be little doubt that these are the "tetrads" of authors, though in *Crepidula* they are not always 4-lobed. As these chromosomes increase in size a hole appears through the middle, between the lobes. There are also found circular or elliptical rings which may be completely closed or may be open on one side; also dumb-bell and cross-shaped bodies. All these forms are represented in text fig. I; all the 2-part chromosomes are shown in the first line (A), the 3-part ones in the second line (B), and the 4-part one in the third (C). These forms are grouped according to evident resemblances merely and it is not certain that they always stand in the genetic relations indicated. For example, A, 4 and 5 may give rise to B, 6 and 7; B, 4 and 5 may be only variations of C, 3 and 4, etc. In all cases, however, the short chromosomes of the early prophase give rise to rod-shaped or elongated chromosomes in the metaphase. In some cases (e. g. line A) this is probably accomplished by these chromosomes becoming ring-shaped and by the opening of these rings on one side. If the ring shows no thickenings (A, 2 and 3) a rod-shaped chromosome is formed by its opening, which later becomes dumb-bell shaped (A, 4 and 5); if it shows three thickenings (B, 1–4) it gives rise when opened to a rod with a thickening at each end and one in the middle (B, 5). The 4-part chromosomes (C, 1) are frequently drawn out into cross-shaped ones; these crosses usually have a hole through the middle and each arm of the cross is split lengthwise from this hole nearly to the tips (C, 2 and 3). In later stages the

arms which lie in the spindle axes lengthen, while the transverse arms shorten, the hole disappearing; in this way chromosomes are formed with three enlargements (C, 4 and 5) similar in all respects to those described above (B, 4 and 5). The resemblance of these two forms is so close that it is difficult to explain the differences in their mode of origin. It is possible that forms, such as those shown in B, 4 and 5, are really crosses with short transverse arms, the tips of all four arms being bent toward each other until they nearly or quite meet.

The striking differences in the shapes of the chromosomes of the prophase is continued into the metaphase where at least three distinct types may be recognized as shown in text fig. I, lines A, B and C. In the late anaphase, however, all come back to a cubical or tetrafoil condition; a hole is usually present through the middle of these as in the prophase.

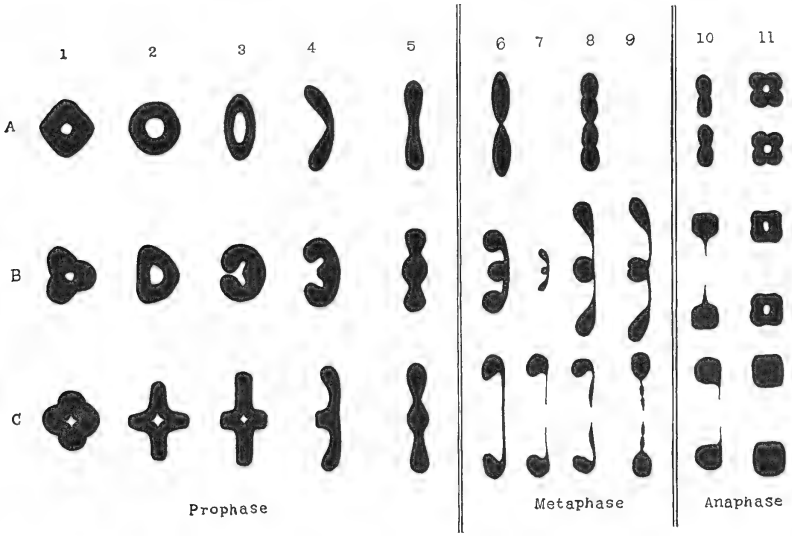

Fig. I.—Chromosomes of the First Maturation Division of Crepidula.

Various authors have called attention to the variety in the form of the chromosomes of the first maturation division (v. Klinckowström '96, Van der Stricht, '98, Foot '98, Lillie '98, Griffin '99). Foot and Lillie figure 3-part chromosomes in the metaphase of the first maturation of *Allolobophora* and *Unio* entirely similar to those in *Crepidula*, and Lillie shows these chromosomes split longitudinally, as they must be, if formed from crosses as shown in text fig. I, line C.

It is difficult to say whether these differences in the shapes of chromosomes mean much or not. On the one hand it is possible that all the chromosomes of a

given mitosis cannot be reduced to a single type; that their differences in shape indicate differences in material substance, and that different chromosomes may therefore represent different heritable qualities. On the other hand these differences in the shapes of chromosomes are generally limited to the first maturation division; they are rarely found in the second maturation and only to a limited extent in the cleavage. Furthermore, there are many evidences that the shapes of chromosomes are conditioned by their linin sheaths, and that the chromatic substance which fills the sheath is of a semi-fluid of viscid character. Thus in the metakinesis of the first maturation, it is always found that the chromosomes have enlarged ends toward the poles of the spindle, and that they are drawn out into thin connecting threads in the equator. In this region the chromosomes are frequently moniliform in shape (text fig. I, C, 8 and 9 and Plate I, fig. 13), and cross sections through the equatorial region of these elongated chromosomes shows many of the latter surrounded by a clear zone, which is bounded by a dark line (Plate I, fig. 12*a*). This clear zone is entirely lacking in sections through the enlarged ends of the chromosomes. In fig. 12*a* one chromosome lies entirely outside of the spindle substance, and yet it is surrounded by this clear zone; this zone and its outer dark boundary is not therefore a mere expression of the absence of spindle substance around the chromosome, but is a structural peculiarity of the chromosome itself, and probably represents a linin sheath, which is separated from the contained chromatin in the equator, but is entirely filled by the chromatin at the poles. After the complete division of this thread of chromatin and its withdrawal into the enlarged ends of the daughter chromosomes, the linin sheath may still be seen for a long time connecting the latter together, and constituting a connective fibre.

The chromosomes grow continually during the early stages of the first maturation division and reach their greatest size in the metaphase when each is from two to twenty times the size of the largest granule present in the germinal vesicle (cf. figs. 3 and 12). After this stage they do not appear to increase in volume. The great differences in the size of chromosomes in the same spindle is almost as striking as their differences in shape; for example, the volume of the largest 3-part chromosome in Plate I, fig. 12 and text fig. I, B, 7 and 8, is about fifteen times that of the smallest; I am unable to say, however, whether this difference in the size of chromosomes is the same in all eggs or not. I do not remember that any one has recorded such enormous differences in the size of chromosomes as are here described. Montgomery ('98) says that the chromosomes of one of the spermatocytic divisions in *Pentatoma* vary greatly in volume, the largest sometimes having six times or more the volume of the smallest. In no other mitosis in *Crepidula* is there such variety in the size of chromosomes, and nowhere else are there such differences in shape.

The number of chromosomes in the first maturation division is thirty, as I have determined by a careful study of the entire mitotic figure, as well as by cross sections through the equatorial plate. Such a cross section is represented in Plate I, fig. 12*a*, and the whole number of chromosomes is there shown. This is undoubtedly the

reduced number of chromosomes; I have been unable to count with accuracy the number present after fertilization, but it is evidently about sixty.

In the early anaphase of the first maturation, the daughter chromosomes are either dumb-bell shaped, or cubical or spherical masses, frequently with a slender process of chromatin running from each daughter chromosome toward the other, figs. 13–15 and text fig. I. A little later each chromosome becomes cubical or quatrefoil in shape, and this form persists, and is universal until the metaphase of the second maturation division, when all become 4-parted, figs. 16–31 and text fig. II, and then cross shaped exactly as in the first maturation. In this case, however, the arms of the cross are not split longitudinally, but the separation takes place between the arms, so that in the metakinesis two of the arms or spherules, in the form of a dumb-bell, go to one pole and two to the other, text fig. II, 5 and 6. In the anaphase of the second maturation, these dumb-bell or rod-shaped chromosomes again become cubical or spherical, as in the anaphase of the first maturation (Plate II, figs. 32, 33 and text fig. II, 8).

The cubical chromosomes at the close of the first maturation are about half as large as the fully-formed chromosomes in the metaphase of that division, while those in the anaphase of the second maturation are about one-half the size of those in the prophase of this division, *i. e.*, the volume of each chromosome at the end of the second maturation division is about one-quarter that of the fully-formed chromosome in the metaphase of the first maturation. There has been therefore no growth of the chromosomes after the metaphase of the first maturation. The number of chromosomes in the second maturation division is the same as in the first, viz. thirty, and the same number is left in the egg after the second polar body has been formed.

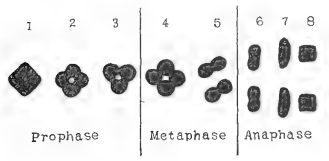

FIG. II.—Chromosomes of the Second Maturation Division of Crepidula. (In the reproduction this figure has been reduced more than fig. I.)

It is especially noteworthy that in the prophase and anaphase of both maturation divisions the chromosomes are cubical or quatrefoil in shape. In the metaphase of the second maturation, figs. 27–31 and text fig. II, the chromosomes look like typical "tetrads" and they would undoubtedly be called such if they occurred in the first maturation.

Similar 4-parted chromosomes in the second maturation are figured by v. Klinckowström ('96) in *Prostheceraeus,* by Van der Stricht ('98) in *Thysanozoon,* and by Byrnes ('99) in *Limax.*

In *Crepidula* it is impossible to say whether the plane of division of the chromosomes is the same or is different in the two maturation divisions. At the beginning and at the end of both divisions the chromosomes are cubical or quatrefoil in shape, and one might as well speak of the "longitudinal" or "transverse" division of a cube or sphere as of these chromosomes. It is impossible, therefore, to determine whether or not reduction in the sense of Weismann takes place in this case.

Griffin points out that the division of the tetrad in *Thalassema* and *Zirphæa* is unlike that in the Copepods, in that in the former each spherule of the tetrad becomes an arm of a cross and that these arms then split longitudinally, whereas in the Copepod type two entire spherules separate from the other two. The former he calls a "spurious tetrad" (cross form), the latter a "tetrad of the Copepod type." In *Crepidula*, just as in *Thalassema* and *Zirphæa*, the tetrads are of the "spurious" type in the first maturation, whereas in the second maturation we have chromosomes which, in every respect, resemble tetrads of the Copepod type.

In the late anaphase of the second maturation the chromosomes which remain in the egg become vesicular and fuse together to form a few vesicles with large granules of chromatin on their walls, Plate II, figs. 34–35. Finally all of these vesicles fuse into one, as is shown in fig. 36, *et seq.*

2. Centrosomes and Central Spindles.—The earliest stage at which centrosomes have been seen was in an egg from the oviduct, not yet fertilized, fig. 2. In this egg the centrosomes are already present as two minute bodies, in contact with the nucleus and without any apparent central spindle or polar radiations. In fact, because of the absence of these radiations it is impossible to be certain that the two granules shown in fig. 2 are really centrosomes. In other eggs from the oviduct, figs. 3–7, into which a spermatozoon has penetrated, the centrosomes are larger, a central spindle is present and polar fibres are abundant. I have been unable to determine whether this central spindle arises as a centrodesmus (Heidenhain) or whether its fibres grow out independently from the two centrosomes and afterwards unite to form the spindle. In general it may be said, that the formation of the mitotic figure usually begins with the entrance of a spermatozoon into the egg.

In the prophase of the first maturation the centrosomes are minute densely staining points; they grow larger as mitosis advances, and in the stages immediately preceding the metaphase, figs. 8, 8*a*, 11, and text fig. III, they are more or less irregular in shape, and when deeply stained and strongly destained with the iron-alum-haematoxylin of Heidenhain, they may be seen to contain a central clear area. Around this clear area the dense walls of the centrosome are thickened in places and may, perhaps, represent large granules in contact with one another, as Lillie has found to be the case in *Unio*. In the prophase of the second maturation, the centrosomes are so small that I have found it impossible to make out their structure with certainty, but they are in many cases slightly irregular in form (cf. figs. 27–31), from which I conclude that their structure is the same as in the prophase of the first maturation.

In the metaphase of the first maturation (fig. 12), the centrosomes are spherical bodies about 1 μ in diameter. They contain a central area which stains faintly, around which is a thick, dense zone which corresponds to the irregular or granular zone of the prophase; in the metaphase, however, this zone is perfectly regular and gives no indication of being composed of granules as in the preceding stage.

In the anaphase of the first maturation the centrosomes become large hollow spheres, the peripheries of which stain deeply while their central areas remain

clear. Within the central area a faintly staining body becomes visible, which, in its turn, becomes a hollow sphere (text fig. III, *e* and *f*); this is the "corpuscle central" of Van Beneden ('87), or the "centriole" of Boveri ('95).[1]

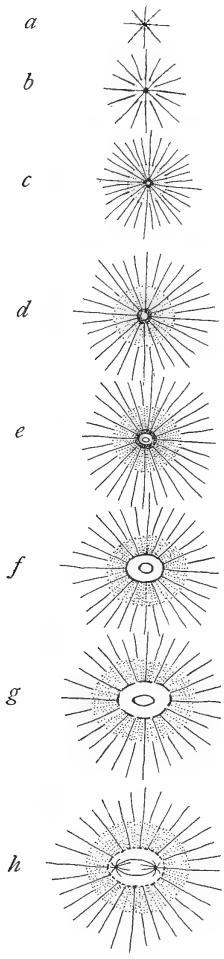

Fig. III.—Centrosomal Cycle in the Maturation of Crepidula.

Up to this stage the centrosomes at the two poles of the maturation spindle are identical in size and structure. When, however, the outer pole of the spindle comes into contact with the egg membrane, the sphere and centrosome at this pole become flattened, figs. 14–16, though the centrosome still shows its dark periphery, its central clear area and its central corpuscle.[2] In the late anaphase the outlines of the centrosome at the outer pole are marked by a layer of granules, while within the central clear area is the elongated central corpuscle, fig. 16*a*. Finally, after the complete separation of the first polar body, the granular outlines of this centrosome disappear, though the central corpuscle, or rather the centrosomes and central spindle to which it gives rise, are still found within it (Plate II, figs. 22, 28*a*, 29, 34, 36).

The centrosome at the inner pole of the spindle continues to enlarge until it reaches a truly astonishing size, becoming fully 4 μ in diameter. Its peripheral layer is at first a solid zone of deeply staining material. In later stages this zone breaks up into plates, figs. 16–25 and text fig. III, and still later it appears as a ring of close set granules.

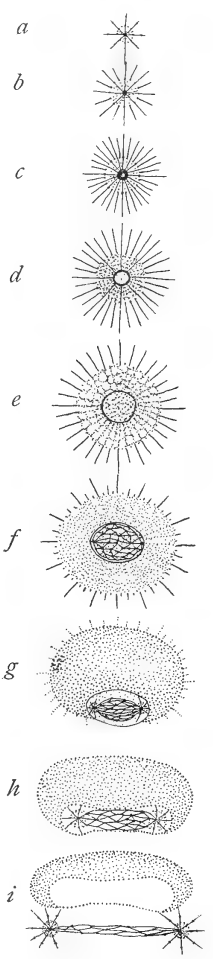

Fig. IV. Centrosomal Cycle in the Cleavage of Crepidula.

[1] Boveri ('01) denies the general homology of his "centriole" with the "corpuscle central" of Van Beneden. There can be no doubt that the structure in question in *Crepidula* is homologous with Boveri's "centriole."

[2] Van der Stricht ('98) has observed a similar flattening of the sphere and centrosome against the egg membrane in the first maturation of *Thysanozoon*.

The central corpuscle, which is shown in figs. 13 and 14 as a faintly staining, hollow sphere, soon becomes elliptical in shape, fig. 15. At the poles of this ellipse its walls grow thicker and stain deeply. These thickened points become the centrosomes of the second maturation spindle, while the remainder of the ellipse forms the central spindle[1] (cf. figs. 14-16*a*, and text fig. III, *g*, *h*). The daughter centrosomes and central spindle lie within the mother centrosome; the outlines of the mother centrosome then disappear and the new amphiaster lies free in the granular remains of the sphere.

During this metamorphosis the centrosome undergoes great changes in its staining qualities; in the prophase and metaphase it stains deeply with hæmatoxylin; as it enlarges, however, the peripheral portion only takes hæmatoxylin, while the central part takes plasma stains; finally, in the late anaphase, even the peripheral portion takes plasma stains.

At no time during this metamorphosis do the astral radiations penetrate the centrosome. As long as they can be seen they remain attached to its surface, and even after the new amphiaster has arisen within the mother centrosome, the rays are still centered on the figure as a whole, figs. 25–28, and text fig. III, *g* and *h*. The new radiations which appear around the daughter centrosomes develop *de novo*, as MacFarland ('97) and Griffin ('99) maintain.

Up to the time when the second polar body is cut off, the history of the centrosomes during the second maturation is similar to that already described for the first; at the beginning of division they are minute granules, as the division advances they become larger, and in the anaphase are large hollow spheres.

After the second polar body has been formed, however, the centrosome which remains in the egg becomes a very large sphere filled with many coarse granules and with a boundary layer of close-set granules, from which, in some cases, polar fibres proceed, figs. 34–36. I have never seen a peculiarly large granule which might be regarded as a centriole within this centrosome, nor have I seen the formation of a central spindle as at the close of the first maturation. On the other hand, the formation of a large number of granules within the centrosome is a phenomenon which occurs in the telophase of every cleavage (text fig. IV), and seems, therefore, to be the more usual process. It seems probable, therefore, that the particular manner in which the daughter centrosomes and central spindle arise within the mother centrosome at the close of the first maturation, is a modification of the more typical process shown in the cleavage, due, perhaps, to the entire omission of a resting stage between the two maturation divisions. In the maturation, there-

[1] In view of the remarkable resemblance of this stage to a corresponding stage in the division of the "centrosome" in *Diaulula* (see MacFarland's figs. 36, 37, *et seq.*) it may be supposed that the vesicular body which I have called the "central corpuscle" is really the centrosome and that the surrounding body is only the inner zone of the sphere. Fortunately, however, the outlines of the centrosome are so perfectly distinct and its history, as shown in my preparations, so continuous, that there can be no question as to its identity in this case. The outlines of the old centrosome remain until after the central corpuscle has given rise to a perfect spindle within it; so that in *Crepidula*, and several other gasteropods which I have studied, the new centers and central spindle arise from the central corpuscle and not from the entire centrosome as in *Diaulula*.

fore, the centrosomes, like the chromosomes, undergo an unusual type of division. The centrosome which goes into the second polar body completely disappears.

The egg centrosome is surrounded by a sphere with grows with the growth of the centrosome until it becomes very large and is filled with faintly staining granules which are held in a delicate reticulum, fig. 36. In some cases the outlines of this sphere are very distinct, in others more faint, but in all cases there are strongly marked polar fibres, which run from the periphery of the sphere for a considerable distance through the egg; some of these fibres may also be traced into the spheres where they appear to break up into rows of granules. In stages later than fig. 36 I am unable to recognize the egg centrosome; its granules merge with those of the surrounding sphere and its outlines are no longer visible.

In structure and metamorphosis the centrosomes of the maturation divisions of *Crepidula* are very similar to those described by MacFarland ('97) in *Diaulula*, Lillie ('98) in *Unio*, Vejdovsky and Mrazek ('98) in *Rhynchelmis* and Van der Stricht ('98) in *Thysanozoon*. The further consideration of these centrosomes will be deferred until after the description of the cleavage.

3. Polar Rays, Spindle Fibres and Spheres.—At the time of the entrance of the spermatozoon into the egg, figs. 3 and 4, the centrosomes are surrounded by polar fibres and the nuclear membrane is indented in the region of each centrosome. A large number of fibres, forming a cone or half spindle, can be traced from each centrosome to the indented portion of the nuclear membrane. Within the nucleus the linin threads, with their attached chromatin granules, are arranged in the same radiating lines as these fibres and form an intranuclear continuation of them, figs. 3 and 4.

In early stages of this division both the extra- and intra-nuclear portions of the spindle consist of branching and anastomosing threads, along which are ranged oxychromatin granules. These two groups of fibres, *i. e.*, those inside and those outside of the nucleus, are so essentially alike that I cannot doubt that both are derived from the same substance, viz., the linin and oxychromatin of the nucleus, in which case the extra-nuclear portion of the spindle must be formed from nuclear constituents which have escaped from the nucleus at the indented areas mentioned above.

The polar fibres also consist of threads along which granules are attached, figs. 4, 5 and 6. In the first maturation they closely resemble the spindle fibres, and, like them, may be derived from the achromatic nuclear substance. These granules are rarely arranged in concentric spheres as Heidenhain, Drüner and Lillie have found them. As mitosis advances, both polar and spindle fibres become smooth. There can scarcely be any doubt that this is accomplished by the transformation of these granules into the substance of the fibre (cf. Boveri, '88, p. 80, Wilson '95, Griffin '99). Again, in the dissolution of the spindle one frequently observes that the fibres become varicose, as in the early stages of mitosis, while the portion of a fibre between granules becomes less and less prominent, figs. 16*a* and 33. I have been unable to observe these same varicosities on the fibres of the central spindle in the maturation divisions, but they can be seen in the central spindles which are found during the cleavage of the egg (Plate IV, figs, 74, 75 and 76, and text fig. IV).

The infolding of the nuclear wall mentioned above must, of course, be accompanied by the escape of some substance from the nucleus. Coincidently with this infolding of the nuclear membrane the polar fibres and extra-nuclear spindle fibres become longer, stouter and granular. At the same time the spindle and the sphere surrounding the centrosome stain more deeply, owing to the presence of an interfilar substance, which stains like nuclear sap.[1] The nuclear membrane then completely disappears, but the nuclear contents preserve for some time the outline of the nucleus and can easily be distinguished from the surrounding cytoplasm because of greater affinity for stains, figs. 6 and 7. The entire mitotic figure, with the exception of the polar systems, lies within this granular area and the enormous growth of the spindle is at the expense of this intra-nuclear substance. Not all of the achromatic substance of the germinal vesicle is confined to the spindle and the polar systems, a large part of it passes directly into the cytoplasm, which is increased in quantity after the nuclear membrane dissolves.

Both the aster and the spindle are plainly composed, during the early stages of mitosis, of two constituents, viz., fibres with their attached granules and an interfilar substance. Between and around the spindle fibres, both in the first maturation and in all subsequent divisions, there is a homogeneous interfilar substance which colors deeply with plasma stains. This substance is sharply delimited from the surrounding cytoplasm, as is shown in figs. 12 and 12*a* and also in later stages of both the maturation and cleavage.

A cross section through the equator of the spindle in the metaphase, fig. 12*a*, shows the interfilar substance of the spindle as a homogeneous mass, staining deeply with orange or eosin, and with stellate radiations running out into the cytoplasm in all directions. These radiations around the equator are shorter, blunter and more irregular than those at the poles. They are probably caused as follows: in the formation of the definitive spindle there is a general elongation of the linin reticulum in the direction of the spindle axis and a contraction of the reticulum at right angles to that axis; at the same time there is a condensation of the interfilar substance, the more fluid karyolymph being squeezed out of the spindle. In this equatorial shrinkage some of the linin threads probably remain attached peripherally and thus cause the stellate radiations.[2] The chromosomes lie within this interfilar substance, though occasionally one is found just outside of it, fig. 12*a*, and they are scattered through the entire thickness of the spindle, and not merely in a wreath around the periphery. There is, therefore, strictly speaking, no "central spindle" in *Crepidula* as contrasted with a "peripheral spindle," but the fibres of the two must be intimately commingled.

[1] Drüner calls attention to the fact that the nuclear membrane is dissolved at points opposite centrosomes and that coincidently the rays grow stronger; and R. Hertwig ('99) has observed in *Actinosphærium* that nuclear material probably escapes into the plasma cones, since the latter stain more deeply about the same time that the nucleus shrinks in size.

[2] Similar radiations around the equator of the spindle have been figured by Korschelt ('95, figs. 131, 139), Mead ('98, fig. 18), and Gardiner ('98, figs. 28, 34).

An interfilar substance, which is to all appearances similar to that of the spindle, surrounds the centrosomes and radiates along the polar fibres, so that in all middle stages of mitosis it is difficult to recognize the polar fibres and spindle fibres when once they are surrounded by it.

In the later stages of mitosis this interfilar substance moves to the poles of the spindle, again allowing the spindle fibres to be seen distinctly; it also moves inward toward the centrosome, leaving the polar fibres sharply marked, fig. 22, and thus aggregated, from the spindle and polar rays, forms a sphere with rather indefinite outlines. This sphere differs notably in character from that which is found in many other animals, e. g., the *outer sphere* of *Unio* (Lillie '98) and the *couche corticale* of *Thysanozoon* (Van der Stricht '98). The latter are clear zones of definite outline, with faintly staining astral rays running through them; in *Crepidula*, on the other hand, this zone is indefinite in outline until the late anaphase or telophase, and is even then not so sharply bounded as Lillie and Van der Stricht have shown it; further, instead of being a zone which is clearer than surrounding parts, it is denser and more deeply staining. The spindle fibres and polar rays can be traced through this sphere to the centrosome in early stages of mitosis, but in middle stages fewer radiating fibres can be seen in it (cf. figs. 3–8*a* with figs. 11–16). In later stages again polar rays can be traced through it to the centrosome (cf. figs. 22–24 and 34–36).

The origin of this interfilar substance is difficult to determine. In the aster it seems to be principally derived from hyaloplasm (interalveolar substance) of the cell body, which is aggregated toward the centrosomes, the larger alveoles of the cytoplasm and the yolk spherules being crowded out from the centrosome as the interalveolar substance moves in toward it. In the spindle, on the other hand, the interfilar substance seems to be formed in large part from achromatic material of the nucleus; such interfilar substance exists before the nuclear membrane is broken, though it is at this stage much less dense than in the fully formed spindle. When the nuclear membrane dissolves at the poles this substance escapes into the extra nuclear spindle and spheres; it is quite possible that at the same time there may be an invasion of the spheres and spindle by hyaloplasm from the cell, this double movement being in the nature of a diffusion in both directions. The fact that the interfilar substance is denser than either the nuclear sap or hyaloplasm may perhaps indicate that it is a new substance formed by a combination of the two. While this suggestion as to the origin of the interfilar substance accords well with all my observations as to its character and movements, it cannot be considered as more than a suggestion.

The form and size of the first maturation spindle varies greatly in different phases. From the prophase to the metaphase it increases in length and diameter, becoming most stout in the metaphase; in the early anaphase it continues to increase in length, becoming about as long as the radius of the egg, figs. 11–14, and at the same time it grows very slender; finally, in the late anaphase it again shortens, becoming stouter, until it is not more than one-half as long as in the metaphase or early anaphase, figs. 15 and 16, and at the same time the chromosomes are pushed

right into and through the sphere until they come into contact with the cell wall. In no other mitosis is there such a shortening of the spindle; in fact, in all other divisions, with the possible exception of the second maturation, the spindle continues to grow longer throughout the whole of the mitosis. A similar shortening of the first maturation spindle has been observed in *Ascaris* (Boveri '87), *Branchipus* (Brauer '92), *Ophryotrocha* (Korschelt '95), *Myzostomum* (Wheeler '95), *Cerebratulus* (Coe '99), *Polychærus* (Gardiner '98), *Axolotl* and *Triton* (Carnoy and Lebrun '99, see their figs. 110 and 112). The principal cause of this shortening is to be found in the peripheral movement of the mitotic figure, as will be described in Part II; its chief result is the production of a much smaller polar body than would be possible if the spindle maintained its maximum length throughout the later stages of division. At the time of its greatest length the first maturation spindle is about one-half as long as the diameter of the egg, and since the division of the cell body always takes place through the middle of the spindle, the first polar body would have a diameter one-quarter that of the egg were it not for this shortening of the spindle.

I agree with G. Niessing ('99), that the shape of the spindle, *i.e.*, whether it is stout or slender, is due to the quantity and location of the interfilar substance, but this depends upon the degree of contraction of the linin reticulum. Both reticulum and interfilar substance are widely distributed through the nuclear cavity in the early prophase, and at this stage the spindle is very stout; in later stages, as the reticulum contracts and the interfilar substance passes to the poles, the spindle grows slenderer. In the late anaphase, when the spindle becomes shorter, it again grows stouter, figs. 15 and 16.

The second maturation spindle arises within the centrosome left in the egg at the close of the first maturation. At first it occupies but a small part of the cavity of this centrosome, but it grows rapidly until it fills the whole of it. The outlines of the mother centrosome then disappear and the spindle lies free in the sphere substance. Here it grows rapidly in size, but never becomes more than half as long as the first maturation spindle, though it is relatively stouter. Its mantle fibres are not formed directly from a linin reticulum, since there is no vesicular nucleus, though they may possibly be formed from nuclear material which escaped from the germinal vesicle at the previous division.

During the prophase, the direction of the first maturation spindle bears no constant relation to the egg axis. It may lie obliquely or even at right angles to that axis, figs. 9 and 10, but ultimately it moves into a radial position, fig. 12, *et seq.*

The direction of the second maturation spindle, like that of the first, varies greatly, though in all cases it ultimately becomes approximately radial. As in *Physa* (Kostanecki and Wierzejski '96), the outer pole of the second maturation spindle lies at the very point where the mid-body (*Zwischenkörper*) of the first maturation spindle was formed. The second polar body is given off immediately under the first, so that the latter becomes separated from the surface of the egg and remains mounted upon the former. This happens irrespective of the initial direc-

tion of the spindle, which always ultimately turns so that one pole lies immediately under the first polar body. If one may judge by the figures of many authors, this must be a phenomenon which occurs among a large number of animals.

4. Polar Bodies.—Finally, the outer half of the mitotic figure, with a small amount of surrounding cytoplasm, protrudes from the general surface of the egg. The furrow separating the first polar body begins to form at the periphery and proceeds toward the middle of the stalk connecting the polar body with the egg. In some cases the spindle seems to retain its full diameter, even when the cytoplasm has been completely constricted by the dividing furrow, fig. 22, as has also been observed by Kostanecki and Wierzejsky ('96) in *Physa*. Afterward, the spindle itself becomes constricted in the middle, fig. 23; and the constricting ring of darkly staining substance finally cuts the spindle in two and itself becomes a spherical mid-body. During and after the separation of the first polar body, one first becomes aware of the fact that there is an egg membrane, which takes no part in the constriction, but is lifted from the egg by the polar body, Plate II, figs. 22, 23, 28, 30 and 31.

The second polar body is smaller than the first and is separated from the egg in the same way as the first, a mid-body being formed, as shown in figs. 34 to 41. This mid-body is larger and persists longer than that of the first maturation, as Mark and Kostanecki and Wierzejski have also observed. When fully developed, it consists of a central granule and two surrounding spheres, the inner one small, dense and sharp in outline, the outer one large, less dense and indistinct in outline. The remains of the spindle can be seen running through this outer sphere as two cones, their apices being in contact with the inner sphere and their bases with the two nuclei.

The first polar body divides by mitosis into two, figs. 27, 28*a*, 32, 34 and 36, and each of these may subdivide amitotically into a large number of cells, some of which are unequal in size and recall the macromeres and micromeres of developing ova, figs. 41, 45, 61, 69, 73 and 81. I have never seen the second polar body dividing.

II. Fertilization.

1. Entrance of Spermatozoon.—Copulation occurs only at long intervals, perhaps once in the life time of a female, and the spermatozoa are stored after copulation in a tubular outgrowth of the uterus. Ova and spermatozoa meet in the uterus, and here the entrance of the spermatozoon occurs, though the later stages in the approach of the egg and sperm nuclei do not occur until after the capsules have been formed and deposited. In the examination of thousands of eggs taken from the egg capsules I have never found one which was unfertilized and very few into which more than one spermatozoon had entered.

A mature spermatozoon is shown in fig. 17; there is a relatively large head with pointed apex, separated, in preserved material, by a clear space from the tail. I am inclined to regard a minute, darkly staining cap which covers the posterior end of the head as the middle piece. It is extremely small and appears to contain no

archoplasm.[1] A spermatozoon enters the ovum almost immediately after it reaches the uterus and while the germinal vesicle is still intact, fig. 3, *et seq*. The sperm may enter at any point on the surface of the egg, except within a small area immediately surrounding the animal pole; usually, however, it enters near the vegetal pole. Polyspermy is exceedingly rare; one sometimes finds several spermatozoa attached to an egg, and in a few cases two spermatozoa may be found penetrating the egg membrane or lying just within it, fig. 10, but only on one or two occasions have I seen two well-developed sperm nuclei within one egg. The pointed head of the spermatozoon bores through the egg membrane, figs. 18, 19, 20, though the tail does not enter. After the sperm head is well through the egg membrane several granules are found just behind the head; these are probably derived from the middle piece. Their number and arrangement is variable, but there are always more than two, so far as I have observed, and they are never grouped at the poles of a spindle. After its entrance, the head occupies such positions as to justify the belief that it turns around, as is known to be the case in many other animals (cf. figs 18, 19, 20, 21).

Foot ('94 and '97) has described in *Allolobophora* a number of dark round bodies which stain as intensely as the sperm head itself, and which lie on each side of the head or at its posterior end. These she calls the *sperm granules* and suggests that they may be formed from metamorphosed archoplasm. They are not constant in appearance and may be the result of degeneration.

Byrnes ('99) has also observed in *Limax* a number of darkly staining granules which accompany the sperm head. She suggests that they are derived from particles of chromatin constricted off from the sperm nucleus. Later they disappear and become scattered through the cytoplasm of the egg.

In the main the resemblance of these "sperm granules," both of *Allolobophora* and *Limax*, to those which I have observed in *Crepidula*, is striking enough. I cannot believe, however, that they are degeneration products in *Crepidula* and for that reason, among others, have not adopted Foot's name for them.

2. The Germ Nuclei.—Immediately after the sperm head has entered the egg it is seen to be a pointed rod with three constrictions and four enlargements, having much the same size and shape as one of the 4-part chromosomes found in the metaphase of the first maturation division, fig. 18. It soon grows shorter and thicker and becomes dumb-bell shaped, fig. 20, then nearly spherical, figs. 10, 21, and then irregular or amœboid, figs. 39, 40. Up to this stage it has remained chromatic throughout, but from this time forward spaces filled with achromatic substance appear within it and it begins to grow vesicular. V. Klinckowström ('96) and Van der Stricht ('98) have observed a similar transformation of the sperm nucleus in *Prosthenceraeus* and in *Thysanozoon*, the sperm head being first moniliform, then spherical, then vesicular.

[1] Byrnes ('99) finds no middle piece in the spermatozoon of *Limax* and suggests that it may possibly be surrounded or overgrown by the sperm head.

The egg nucleus is formed by the fusion of the chromosomal vesicles left in the egg at the close of the second maturation, as described on p. 14.

The further changes of the germ nuclei may now be briefly followed as far as the prophase of the first cleavage. The developments of both germ nuclei are entirely parallel, so that a single description will serve for both. As soon as the vesicular stage of each nucleus is reached the chromatin is found to be stretched through the nucleus in the form of a reticulum, figs. 36–41. As the nuclei enlarge the chromatin takes more and more the form of rounded masses, Plate III, figs. 44, 45 and 46, while the reticulum connecting the masses becomes extremely tenuous and does not stain. In short, there is at first a *chromatin* reticulum, which in later stages becomes a *linin* reticulum with the chromatin aggregated at nodal points. The chromatin masses differ considerably in size, fig. 45, and are at first quite solid. In later stages, figs. 49–53, these masses become hollow spherules. Those spherules which develop into chromosomes become connected together into a linear series, and either remain solid or at least have thicker walls than those spherules which take no part in the formation of the chromosomes. The further history of the chromatin will be taken up under the head of the first cleavage. As soon as the vesicular stage of each germ nucleus is reached there appears within it a single large nucleolus.[1] This persists until a stage when the two nuclei come into contact, fig. 44, when it is usually dissolved in the nuclear sap, though sometimes traces of the nucleoli may be seen in later stages, *e. g.*, fig. 49.

3. Egg and Sperm Asters and Spheres.—The history of the egg centrosome and sphere in the second maturation division has already been considered, pp. 16 and 17. At the same time that the egg aster is being transformed into the enormous egg sphere, figs. 32–36, a sperm aster has appeared and is undergoing a parallel transformation. The various stages in this process occur at approximately the same time in the two, though the sperm sphere and nucleus remain slightly smaller than those of the egg until the nuclei lie near each other. We may now follow in detail the origin of the sperm sphere.

After its entrance the sperm head lies among the yolk spheres in a small quantity of cytoplasm, while the granules derived from the middle piece lie just behind the head. There is at this stage no trace of astral radiations anywhere in the egg, except in connection with the first maturation spindle. The sperm nucleus lies in this position, near the periphery of the egg, without any trace of astral radiations near it, until the anaphase of the second maturation division. At this time the nucleus has become irregular or amœboid in shape and some distance from the nucleus, toward the center of the egg, the sperm aster appears. It is a noteworthy fact that no sperm aster appears until the sperm nucleus begins to absorb achromatic material, and this suggests that the two processes stand in some causal relation to each other. Furthermore, the fact that the two spheres are proportional in size to their nuclei, and that the sperm sphere remains smaller than

[1] Mark ('81) observed in an undetermined species of *Limax* that each of the germ nuclei contained a single nucleolus.

the egg sphere as long as the sperm nucleus is smaller than that of the egg, lends further weight to this suggestion.

The earliest stage in the formation of the sperm aster which I have seen is shown in figs. 39 and 40. I have examined thousands of eggs of earlier stages, but have failed to find a sperm aster in any of them. The aster when first seen is a radiating figure in the cytoplasm, with several dark granules at its center. The number, position and size of these granules is not constant, and in later stages they greatly increase in number and stain less darkly than at first; there can be little doubt that they are identical with the granules derived from the middle piece. The sperm aster with the granules at its center ultimately becomes more rounded in outline and forms a large sphere from which radiating fibers proceed in all directions. This sphere exactly resembles the sphere in contact with the egg nucleus, fig. 41.

From the time of their first appearance each of these spheres lies close to its own nucleus, and they do not wander from these relative positions so that there is no possibility of confusing or mistaking them. During the approach of the sperm nucleus and aster to those of the egg, one or two small accessory asters appear in the egg, usually at some distance from the sperm and egg nuclei (figs. 42 and 43); these resemble the minute asters described by Mead ('98), and Lillie ('98) as "accessory asters." They contain no centrosomes or large granules, and their origin at a distance from the egg and sperm asters shows that they are independent of either of these. These accessory asters are present for a brief period only and then completely disappear.

At no stage in their development do the egg and sperm spheres show the compact and densely staining qualities which the spheres show throughout the cleavage stages; this added to the fact that there is a less perfect separation of cytoplasm and yolk during the fertilization than in the cleavage makes the study of these structures difficult, and this is especially true in the stages just before and after the fusion of the spheres. While designating these structures "spheres," both because of their form and also because of the derivation of the egg sphere from the sphere left in the egg at the close of the second maturation, I would not be understood as positively homologising them with the "outer sphere" or "cortical zone" of authors.

4. Approach of Germ Nuclei and Spheres.—The egg nucleus and sphere remain at the upper pole, immediately beneath the polar bodies, and do not move from this position. The sperm nucleus and sphere move toward those of the egg in a path which is at first directed toward the center of the egg ("Entrance path," Roux), and then toward the egg nucleus ("Copulation path"). If the sperm enters near the lower pole, the course of the sperm elements is nearly straight through the egg from the lower to the upper pole; if it enters at any other point than the vegetal pole, the path is a curved one, the "entrance path" curving more or less sharply into the "copulation path," depending upon the distance of the point of entrance from the vegetal pole. In all cases the sperm elements approach those of

the egg from the lower side, and during the prophase of the first cleavage the germ nuclei usually, though not invariably, occupy the same relative positions, the egg nucleus being above and the sperm nucleus below, figs. 42–55. The positions of the spheres relative to the germ nuclei is not perfectly constant, though the sperm sphere usually precedes the sperm nucleus and the egg sphere lies on the central side of the egg nucleus. The spheres remain distinct during the approach of the germ nuclei, one being quite as evident as the other, and neither showing any trace of degeneration. A number of yolk spherules are carried before the sperm into the protoplasmic area surrounding the egg nucleus and sphere, and thus it happens that several yolk spherules are usually found between the two germ nuclei and spheres, and more or less isolated from the remainder of the yolk. The germ nuclei first come into contact, as shown in figs. 44 and 45, and afterwards the spheres meet, inclosing still some of the yolk between them; the spheres then completely fuse, figs. 45, 46, 47, 49, 50, 51, *FS*.

Before fusion the spheres consist of masses of faintly staining granules, and a more or less distinct boundary line separates them from the remaining cytoplasm; from this boundary a few fibres or rows of microsomes radiate. This boundary line is sharper in some cases than in others, but is always faintly marked. Immediately before and after the fusion of the spheres it can be seen that the coarse granules in the spheres are nodal points in a very delicate reticulum, figs. 45–47 and 49–51. As soon as the spheres have fused, their substance surrounds the nuclei and spreads in a faintly staining mass into the cytoplasm above the nuclei and immediately below the polar bodies. A similar area of darkly stained protoplasm has been observed by Coe ('99) in *Cerebratulus* (see his figs. 23–28), and is said by him to be derived from the germinal vesicle. In *Crepidula* there can be no doubt that this area is derived from the egg and sperm spheres, though these in turn may be derived from material escaped from the germinal vesicle. All this time very faint radiations proceed from the periphery of the fused spheres, figs. 46, 49, 50. In *Arenicola*, according to Child ('98), the germ nuclei, when they meet, are surrounded by an area of reticular cytoplasm from which radiations run into the surrounding substance of the egg. Child regards these radiations as possibly the result of the absorption of liquid by the germ nuclei, while the reticulum, he thinks, may indicate an accumulation of liquid around the nuclei.

In *Crepidula* the spheres are present during the period when the germ nuclei are growing most actively; they lie in close contact with these nuclei and appear to be associated with their rapid growth. I am inclined to regard them as the expression of certain chemical and physical processes, taking place between the nuclei and the cytoplasm, rather than as structures of high morphological significance.

5. Origin of Cleavage Centrosomes.—In several cases I have observed two large granules among the microsomes at the periphery of the spheres, from which stronger radiations proceed into the cytoplasm, but not into the spheres, figs. 47, 50, 51. These granules are but little larger than others in the peripheral layer of the spheres, and the radiations proceeding from them are but a trifle stronger and more

perfectly centered. Nevertheless they are the only structures in the egg at this stage which at all resemble centrosomes, and I believe, though I cannot positively affirm it, that they become the centrosomes of the first cleavage spindle. In a slightly more advanced stage, figs. 48, 52, 53, unmistakable centrosomes are present; they are no larger than the granules of the preceding stage, but the radiations are larger and more numerous, and they proceed in all directions from them. Those radiating fibres which are directed toward the germ nuclei come into contact with the nuclear membrane, which becomes infolded at this point, and at the same time a darkly staining, homogeneous fluid escapes from the nucleus thus forming a cone or half spindle, the base of which is applied to the nucleus, while the apex reaches to and surrounds the centrosome.

As soon as the undoubted centrosomes appear the fused egg and sperm spheres lose their boundaries, and their granules are either dissolved, or are scattered through the cytoplasm, figs. 48, 52, 53. The cleavage centrosomes are from the first independent of each other, and not until a later stage (figs. 54 and 55), is there any trace of a "central spindle" between them; these fibres grow out from each centrosome until they meet and fuse, just as MacFarland ('97) has observed in the first cleavage of *Pleurophyllidia*.

In view of the controversy as to the origin of the cleavage centrosomes in different animals, it is important to know what relation these centrosomes bear to the egg and sperm spheres of *Crepidula*. Unfortunately no conclusive answer can be given to this question since the centrosomes do not appear until after the spheres have fused.[1] There are certain evidences, however, which point to the conclusion that each sphere gives rise to one of these centrosomes. The evidences are the following:—(1) in fig. 45 a number of yolk spherules lie between the egg and sperm spheres which are here entirely separate; in figs. 46 and 47, the principal mass of yolk within the fused spheres probably marks the line of fusion between the two spheres; in fig. 47 a centrosome lies on each side of the principal aggregation of these yolk spherules, and therefore it is probable that one centrosome has arisen from that part of the fused sphere which was the sperm sphere, and the other from the half which was the egg sphere; (2) until the time of fusion each sphere is closely connected with, in fact partially surrounds, its own nucleus. Even after the fusion it can be seen, fig. 46, that a denser portion of the fused sphere is connected with each of the germ nuclei. Now, if the centrosomes arose, one from the egg

[1] Since this was written more recent work on this subject has shown conclusively that centrosomes and spindles may arise separately in connection with each germ nucleus. If the recently fertilized eggs of *C. plana* are put into a 1 per cent. solution of sodium chloride in normal sea water for 4 hours, a perfect karyokinetic spindle, though about one-half the size of the usual cleavage spindle, appears in connection with the egg nucleus, although the latter may be separated from the sperm nucleus by almost the whole diameter of the egg. If the sperm nucleus is small and densely chromatic no spindle is formed in connection with it; if, however, the sperm nucleus has grown until it contains a considerable quantity of achromatic material a perfect spindle may be formed in connection with it also; in such cases the two spindles usually lie close to each other and may form a tetraster. This experiment suggests that the contradictory observations of different investigators on different animals may find an explanation in the varying rates of growth of the germ nuclei within the egg or in slight differences of the environment.

sphere, the other from the sperm sphere, we should expect to find a centrosome in connection with each germ nucleus and with no connecting central spindle between them. This is just what occurs. In figs. 50–51 the two centrosomes are so placed as to suggest that one is related to the egg nucleus and the other to the sperm nucleus, and in figs. 48–53 there can be no doubt about this fact. In no egg examined is there a trace of a central spindle connecting the two centrosomes until after the centrosomes are in their definitive positions and the nuclear membrane is broken down at the poles of the spindle, figs. 54–55. Even though the centrosomes may lie in their definitive positions at an early stage, a thing which sometimes occurs (fig. 52), they are still quite independent, there being no central spindle fibres between them. This evidence, therefore, although not entirely conclusive, is favorable to the view that one of the centrosomes of the first cleavage spindle comes from the egg sphere and the other from the sperm sphere.

Such a conclusion as to the origin of the cleavage centrosomes is at variance with all observations which have been made heretofore,[1] and it is with much hesitation that I bring it forward without being able to demonstrate its truth in the clearest and most satisfactory manner. I have finally determined to publish these observations only after having spent several years in trying to get indisputable evidence upon this point, so far without success. However, the evidence, as far as it goes, points to the conclusion that both egg and sperm spheres contribute to the formation of the cleavage centrosomes.

In view of the fact that, in *Crepidula*, egg and sperm centrosomes and spheres undergo parallel metamorphoses and that both spheres persist until their union, the commonly accepted view that the spermatozoon alone contributes to the cleavage centrosomes seems in this case highly improbable. Further, there is no particle of direct evidence in favor of this view; there is no sperm amphiaster as in many other cases; when the cleavage centrosomes first appear there is no central spindle between them, as would be the case if both were derived from a single sperm centrosome; a centrosome usually appears in connection with each germ nucleus, which is also inexplicable on the supposition that both have come from the spermatozoon. These same facts are equally strong against the supposition that both cleavage centrosomes are derived from the egg centrosome.

On the other hand it is quite possible that both cleavage centrosomes are new formations, *i. e.*, are not directly derived from the egg and sperm centrosomes, but have arisen independently of these and of each other, in the remains of the fused spheres. Apart from the evidence that one centrosome comes from each of the

[1] It most closely resembles the results of Carnoy and Lebrun ('97) on *Ascaris*, though it differs fundamentally from these in that these authors claim that the cleavage centrosomes arise from nucleoli, one of which comes from each of the germ nuclei.

Since the above was written Lillie's (1901) complete paper on the maturation, fertilization and cleavage of *Unio* has appeared, and the account which he gives of the origin of the cleavage centrosomes in that animal is strikingly like my observations as to the origin of these centrosomes in *Crepidula*. In brief he finds that one cleavage centrosome arises in connection with each germ nucleus, that there is no central spindle between them and that they arise near or in the margin of the sphere substance. He does not consider that they are descendants of the egg centrosomes or sperm amphiaster, but that they are egg products of new origin.

spheres, there is no reason to be alleged why both may not be new formations without genetic relationship to egg or sperm centrosomes, except the analogy of the cleavage stages, where a persistence of centrosomes in all stages can be clearly established.

In a former account of the fertilization of *Crepidula* (Conklin '94) I described a form of "Quadrille of the Centers," in some respects similar to that observed by Fol ('91) and Guignard ('91). In this account I expressly stated that I had not seen the centrosomes during the fertilization, but only the egg and sperm "asters." My account of the persistence and approach of both the asters until they come into contact, I am now able to confirm. However, my account of their subsequent division into halves and the union of these halves by pairs to form the cleavage asters was incorrect. Judging by what I have since seen I am convinced that in my former paper I mistook lobulations of the egg and sperm spheres such as are shown in fig. 44, for division of those spheres, and other similar lobulations of the fused spheres, figs. 46–49, for the union of half-spheres to form the cleavage asters.

The present stand of the question of the centrosomes in fertilization is so well known that it demands no extensive treatment here. Following the publications of Fol, Guignard, Blanc and myself, papers on this subject came "fast and furious." Boveri ('95), Wilson and Mathews ('95), Hill ('95) and Reinke ('95), showed that no quadrille occurred in the Echinoderms; Kostanecki and Wierzejski ('96), MacFarland ('97), and more recently Griffin ('99), and Linville (1900), held that it did not occur among mollusks; Guignard's work has failed of confirmation by other writers; Van der Stricht ('95), who held that a quadrille occurred in *Amphioxus,* has been followed by Sobotta ('97), who maintains that there is no quadrille in that animal, and Blanc ('93), who described a form of quadrille in the trout, has been followed by Behrens ('98), who finds that both the cleavage centrosomes in that animal comes from the sperm; and so the quadrille went to its death.

On the other hand Boveri's ('87–92) view that the cleavage centrosomes were introduced by the spermatozoon, and that "*it is the centrosome alone which incites the division of the egg, and is, therefore, the fertilizing element proper*" (Wilson '96, p. 140), was eagerly championed by more than a score of writers; in fact this doctrine was much more cautiously held by Boveri than by many of his followers. However, there has been accumulating a body of evidence to show that the cleavage centrosomes do not, in all cases at least, come from the spermatozoon. Apart from the long known fact that cleavage centrosomes are present in parthenogenetic eggs, many observations have been made on fertilized eggs which tend to show that these centrosomes may come from the egg centrosome or may possibly arise independently of either the egg or sperm centrosomes. I refer particularly to the work of Wheeler, Foot, Mead, Lillie, Child and myself. In almost every case so far observed there is a period, more or less prolonged, during which no centrosomes are visible (cf. Coe, '98, p. 455). In only a few cases is it affirmed that the sperm centrosomes can be traced without a break into the cleavage centrosomes.

So far as the mollusks are concerned there does not seem to be a single case in

which the cleavage centrosomes are undoubtedly derived from the sperm centrosome. As to *Physa*, in which this origin is strenuously maintained, Kostanecki's figures are capable of another interpretation than that which he puts upon them. All of his figures which show the two germ nuclei and the two centrosomes up to the time when the latter have taken their final position at the poles of the nuclei (his fig. 33*a*) show one centrosome in connection with each nucleus and nowhere in these stages is a central spindle shown, except in fig. 30, which shows a single fibre continuous from pole to pole; even in the later stage, fig. 33*a*, there is no central spindle. Further, it is a significant fact that when the egg centrosome disappears the sperm centrosome also disappears (fig. 25–28), while the next stage figured (fig. 30) shows two large and well marked centrosomes, and in fig. 31 one of these lies in close connection with each of the germ nuclei.

In *Pleurophyllidia*, according to MacFarland, the sperm asters and centrosomes disappear completely during the formation of the second polar body and for a relatively long period no centrosomes are present. After the germ nuclei are in contact the cleavage centrosomes appear, and since they frequently occupy positions similar to the sperm centers, the author thinks they are derived from these.

In *Unio* Lillie finds that both egg and sperm centrosomes and asters completely disappear and that accessory centrosomes and asters also arise and disappear. Finally the two cleavage centrosomes arise independantly of each other and of any of their predecessors.

In *Limnæa* Linville finds that both egg and sperm centrosomes disappear for a time, but since the cleavage spindle first involves the sperm nucleus, he concludes that the cleavage centrosomes are of spermatic origin. His figures, however, do not bear out this interpretation; fig. 6 shows the incipient cleavage spindle in connection with what is surely the egg nucleus, though he calls it the fused germ nuclei, (so far as I am aware the germ nuclei do not fuse in any mollusk.) Fig. 18, which is one of the earliest of his figures showing the cleavage centrosomes, shows one in connection with each germ nucleus and with no central spindle between them.

Boveri's figure of *Pterotrachea* ('90, fig. 10), which is so widely copied in the text-books, shows one centrosome in connection with each germ nucleus and no central spindle between the two.

In other groups of animals the evidence in favor of Boveri's hypothesis is by no means conclusive, while much positive evidence has been brought against it. Among *Turbellaria* I know of no single case clearly favorable to this view; (cf. Klinckowström '96), Van der Stricht ('98), Gardiner ('99), Van Name ('99). Coe's ('99) work on *Cerebratulus* affords very good evidence that the sperm centers become the cleavage centers in that animal, and the same is true of *Chætopterus* (Mead '97), and of *Thalassema* Griffin ('99). On the other hand Foot ('97) has shown in a convincing manner that the cleavage centrosomes are new formations in *Allolobophora* and Child ('98) holds the same position with regard to *Arenicola*.

If all these accounts are to be believed, therefore, the cleavage centrosomes may come from the sperm, from the egg, or from both, and it is at once apparant that

processes which vary so much can have no fundamental or general significance.[1] And even if all these accounts are not accepted, they show that the problem is a peculiarly difficult and complicated one and that it is still too early to formulate generalizations with regard to it.

The evidence is certainly very convincing that in some cases both of the cleavage centrosomes come from the sperm, but in other cases the evidence that they do not have this origin amounts to a demonstration. This is shown in the clearest possible manner in phenomena of normal and artificial parthenogenesis in which of necessity the cleavage centrosomes must have their origin in the egg. Boveri recognizes parthenogenesis as an exception to his generalization and indicates that in such cases the egg centrosome may not degenerate. This view presupposes a fundamental difference between amphigony and parthenogenesis such as does not actually exist. It is well known that in certain animals the determining causes of amphigonic or parthenogenetic development are slight differences in extrinsic conditions; for example there is no fundamental difference between the ova of the honey bee which develop parthenogenetically and those which are fertilized, and how purely accidental in this case are the causes which determine whether there shall be parthenogenesis or amphigony. There is no world wide distinction between these two methods of development and the differences as to the manner of origin of the cleavage centrosomes cannot be fundamental. If in some species the egg centrosome is capable of being preserved or reorganized, it is certainly quite possible that in others it may not degenerate at all. There is therefore no *a priore* reason for supposing that Boveri's hypothesis is of general application and, as I have already attempted to show, it is not in accord with all the facts.

Certainly when one looks at the problem of fertilization from a general point of view, when one considers the universality of sexual reproduction, when one reflects upon the multitudes of exquisite adaptations which exist for securing the union of egg and sperm he will be loath to believe that the essential feature of fertilization is the addition of a centrosome to the egg cell or the supplying of a stimulus to its development which is not needed in all cases and can as well be supplied by changes in density, salinity, temperature, etc., as by the entrance of a spermatozoon.

III. Cleavage.

I have already described the cell lineage or what may be called the external phenomena of cleavage in *Crepidula* (Conklin '97) and must refer to that paper for any detailed account of that process. I may be permitted here, however, to recall a few of the more important features in the early cleavage. In this gasteropod, as indeed also in all mollusks, the cleavage is of a peculiarly determinate, *i. e.*, constant and differential, character.

The first cleavage is equal and divides the egg into two blastomeres which are approximately anterior and posterior in position; the second cleavage is also equal, dividing the egg into right and left portions, Plate V, figs. 80–88. Not only are the

[1] See foot-note.

four cells thus formed (A, B, C and D,) equal in size, but they each contain about the same quantity of yolk. Two of the cells (B and D) meet at the vegetal pole in a polar furrow, whereas all four cells usually meet in a point at the animal pole.

From these four cells thus formed three groups or "quartettes" of small cells, without yolk, are cut off (Plate VI, figs. 89–96). These three quartettes (1a–1d, 2a–2d, 3a–3d) form the whole of the ectoblast of the embryo. The fourth quartette (4a–4d) consists of large cells containing yolk and one of the cells of this quartette (4d) is the mesentoblast and gives rise to most of the mesoblast and also to the posterior part of the intestine. The other three cells of this quartette (4a, 4b, 4c) are purely entoblastic. The first division of the first quartette (1a–1d) is very unequal, giving rise to four large "cephaloblasts" and four small "trochoblasts" (figs. 93–96). The latter are peculiar in structure and history, being clear and non-granular as compared with the cephaloblasts; they divide but once and grow to a great size, giving rise to parts of the velum and head vesicle. The first subdivision of the cephaloblasts is also unequal (figs. 97, 98), giving rise to four small "apical cells" and four large peripheral ones which become the "basal cells" in the arms of a cross of ectoblastic cells, which lies with its center at the apical pole and one arm in each quadrant (figs. 99, 100). The first division of the second quartette is nearly equal (figs. 96, 97), while at the second division four small cells arise which forms the "tip cells" in the arms of the cross (figs. 96–100).

Now as contrasted with these external phenomena of cleavage, which are chiefly concerned with cell boundaries, the internal phenomena consist of certain cyclical changes in the nucleus, centrosome and cytoplasm, each cycle being in the main like every other, though often differing in details. It is in these internal phenomena that the causes of determinate cleavage must be sought and to a study of these phenomena we now turn.

No sharp line of demarkation can be drawn between the fertilization and the first cleavage, since the two overlap, to a certain extent, in point of time. For convenience, however, we may consider the fertilization ended and the first cleavage begun when the centrosomes have taken their definitive positions at the poles of the incipient mitotic spindle. Such a stage is shown in figs. 54–55.

1. The Nuclear Changes During Cleavage.—*a. Independence of Germ Nuclei.*[1]—Until the metaphase of the first cleavage the chromosomes derived from the two germ nuclei are plainly separated into two groups, one derived from the egg nucleus, the other from the sperm, figs. 55–56, text fig. V. During the metakinesis no such separation is recognizable, but in the late anaphase the chromosomal vesicles fuse together into two groups and as the daughter nuclei become vesicular a partition wall is left between these groups, fig. 60, and text fig. VI. In the telophase this partition wall gradually disappears, persisting longest on the side of the nucleus next the centrosome, where a groove marks its position, fig. 81; this groove usually disappears at the height of the nuclear "rest" or "pause," but it appears again in the early prophase of the next division and in almost exactly

[1] An abstract of this section appeared in the *Biological Bulletin*, Vol. II, 1901.

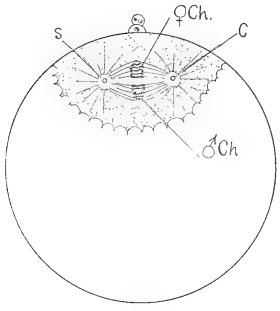

FIG. V.

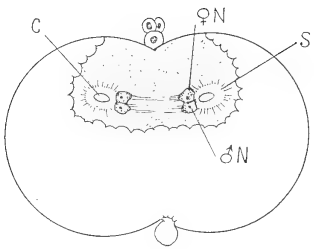

FIG VI.

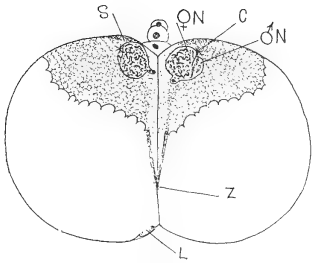

FIG. VII.

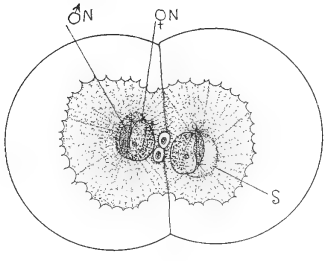

FIG. VIII.

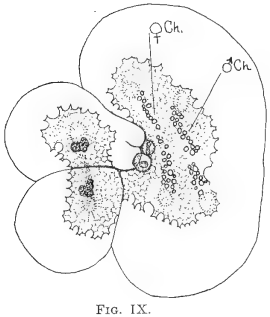

FIG. IX.

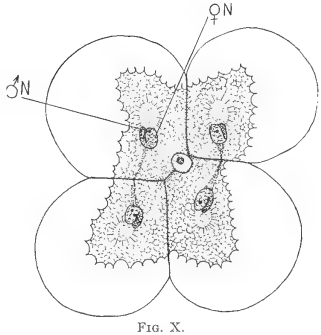

FIG. X.

FIGS. V–X.—INDEPENDENCE OF THE GERM NUCLEI OF CREPIDULA.—The duality of each nucleus is shown in the metaphase and telophase of the first cleavage (figs. V, VI), in the prophase and telophase of the second cleavage (figs. VII, VIII, X) and in an abnormal egg (fig. IX).

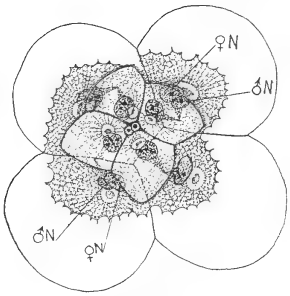

FIG. XI.

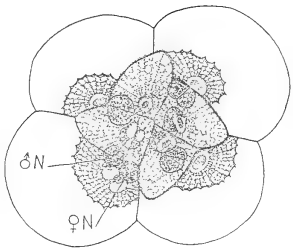

FIG. XII.

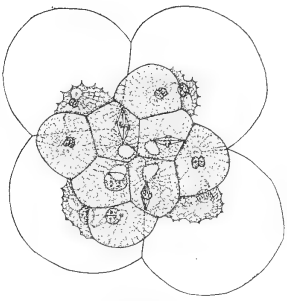

FIG. XIII.

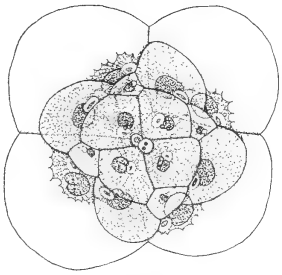

FIG. XIV.

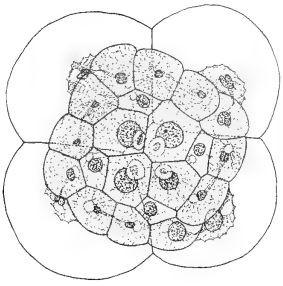

FIG. XV.

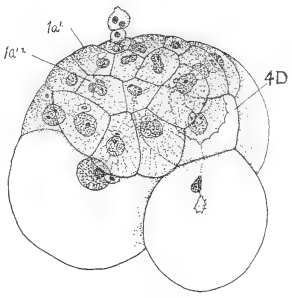

FIG. XVI.

FIGS. XI–XVI.—INDEPENDENCE OF THE GERM NUCLEI OF CREPIDULA.—The dual character of each nucleus is shown especially well in the telophase of each division.

the same position in which it was last seen, fig. 82, text figs. VII and VIII. In this groove the new central spindle for the next cleavage lies and in the following division each half of the double nucleus is divided equally, frequently showing a double chromosome plate in the metaphase. In the succeeding anaphase and telophase each nucleus is again plainly double, being separated by a partition wall into two parts. The dual character of the cleavage nuclei has been observed in the telophase of every cleavage cell up to the 29-cell stage and in many cells up to the 60-cell stage, text figs. V–XVI.

It is very probable that the halves of these double nuclei descend in unbroken continuity from each of the germ nuclei and for the following reasons:

1. In the first and second cleavages the nuclear halves are distinct at all stages except during the metakinesis, and the relative positions of these halves correspond to those of the germ nuclei. Even in later cleavages the relative positions of the nuclear halves indicate that the one lying nearest the animal pole is probably from the egg nucleus and the other from the sperm, text figs. V–XVI.

2. In the first, second, third and fourth cleavages, and probably in all, the central spindle when first formed in the early prophase, lies in a groove between the nuclear halves, and hence in the only plane in which it could lie if the nuclear halves are to be equally divided. Since successive cell divisions in *Crepidula* alternate in direction, it follows, if the plane of nuclear division is always at right angles to the plane of contact between the two halves, that the nuclei or nuclear spindles must rotate at every cycle of division. This actually occurs, as a glance at the text figures will show; the rotation usually occurs in each nuclear cycle before the prophase but sometimes as late as the metaphase.

3. In certain abnormal cases blastomeres are found with two entirely separate nuclei in the resting stage; in other cases two entirely separate mitotic figures lie side by side in the same cell and in one such case, text fig. IX, there are thirty chromosomes in each of these spindles, the same number which is found in each of the germ nuclei.

4. Finally there is always a single nucleolus in each of the germ nuclei before their union, and in all of the cleavages, so far as I have observed, there are two and only two nucleoli present in the telophase, but during the resting period, particularly if it be prolonged, they may fuse into a single one. In view of current teaching with regard to the significance of the nucleolus this persistence of a definite number of nucleoli in each telophase is a somewhat surprising fact and may possibly indicate that there is a persistence of some structure which may act as a center for the formation of the nucleolus in each cell generation.[1] Since the nucleolus itself is dissolved at the beginning of each mitosis, may not some achromatic structure, in which or around which the new nucleolus is formed, persist and be transmitted by

[1] Montgomery ('99) has compiled tables showing the number of nucleoli in the egg cells of 170 or more genera representing almost every phylum in the animal kingdom. As a result of this work he concludes that the number is not constant for a species, that it does not depend upon the amount of yolk, mode of cleavage nor upon the manner of deposition of the egg, and that the facts do not warrant an attempt to explain the factors limiting the number of nucleoli.

division to the two daughter cells? However this phenomenon may be explained, the fact that there is a single nucleolus in each germ nucleus before their union, and that there is a single nucleolus in each half of the dual nuclei during the cleavage, is additional evidence that the halves of these dual nuclei actually represent the germ nuclei.[1]

Such a case as that of *Crepidula* indicates that the apparently single vesicular nucleus of the resting stage may really be double in character, and the fact that out of such a nucleus there may arise in the anaphase and telophase dual daughter nuclei shows that the germ nuclei may still preserve their individuality, though no trace of such separateness may be apparent at other periods. Further, it is possible, even in advanced stages of the cleavage to determine with considerable probability which part of a double nucleus is derived from the egg and which from the sperm, the egg half always lying nearer the animal pole than the sperm half (see text figures V–XVI).

This independence of the germ nuclei during the cleavage of *Crepidula* is fundamentally like the observations of Häcker ('92, '95) and Rückert ('95) on *Cyclops;* here also the germ nuclei do not completely fuse throughout the early cleavage, their independence being most clearly shown in the telophase. Rückert also finds evidence of a similar independence of the germ nuclei in the figures of Fol ('79) on *Toxopneustes* and of Bellonci ('84) and Kölliker ('89) on *Siredon.* Some of these figures referred to furnish very doubtful evidence. For example only one of Fol's figures (pl. VII, fig. 7) shows a dual nucleus, while the figure in Kölliker's textbook (fig. 36) is most probably a case of the indentation of the nuclear membrane opposite the centrosomes, a thing which frequently happens in the early prophase. Bellonci's figs. 1 and 20 show an indentation on one side of the nucleus which may correspond to a division between the germ halves, but none of his figures, with the possible exception of fig. 20, show a fusion of the chromosomal vesicles into two separate groups. Coe ('99) figures an indented nucleus in the telophase of the first cleavage of *Cerebratulus* (see his fig. 40) which probably represents the incomplete fusion of the germ nuclei in this animal. With the exception of Häcker and Rückert, none of the authors named call attention to these indented nuclei or suggest their possible significance, and I think it may fairly be said that *Crepidula* affords the most satisfactory and convincing evidence of the independence of the germ nuclei which has yet been discovered.

These observations are intimately related to the important discovery of Herla ('93) and Zoja ('95) that the egg and sperm chromosomes of *Ascaris* remain independent at least as late as the twelve cell stage, and this discovery was anticipated

[1] More recent experimental work on *Crepidula* egg has shown that when the chromosomal vesicles are prevented from fusing a single nucleolus usually appears within each; in general, one nucleolus is found within each nuclear vesicle, and the fact that two are so generally found in the telophase is probably due to the fact that at this stage the nucleus consists of two vesicles, whereas the more complete fusion of these vesicles in later stages may lead to the formation of a single nucleolus. Such a view would bring the size and number of nucleoli into relation with the size and number of nuclear vesicles present at any stage.

by the hypothesis of the individuality of the chromosomes, first advanced by Rabl ('85) and afterward ably defended by Boveri ('87, '88, '92).

(*b*). *Chromatin.* At the beginning of the prophase of the first and second cleavages the nucleus contains a large number of rounded chromatin granules, which are connected together by a faintly staining linin network, figs. 45–55 and 62–64. These granules are at first solid bodies, but later become hollow spherules,[1] figs. 45–52, and in these stages they all stain alike. Some of these spheres then become united in a linear series, to form the chromosomes, while the others (a large proportion of the whole number) take no part in the formation of the chromosomes and are finally dissolved in the nuclear sap, or are transformed into linin threads. Those spherules which enter into the formation of the chromosomes again become solid and stain more deeply than the others (basichromatin) figs. 50, 51, 62, 63, while those which do not form chromosomes stain less deeply with nuclear stains and gradually come to take plasma stains, (oxychromatin.)

In the prophase of the third, fourth and fifth cleavages the chromatin exists in the form of a reticulum, figs. 70, 71, 74, 75, and not in the form of separate spherules. In the rest preceding the prophase, however, this reticulum is formed of chromatin spherules as in the first and second cleavages, though these spherules are never so evident in later cleavages as in the first two. Some of the threads of this chromatin reticulum become chromosomes; others which show that they are composed of granules, fig. 70, stain much less deeply with nuclear stains, finally taking plasma stains only, and have no part in the formation of chromosomes, but are dissolved in the nuclear sap, or are transformed into linin.

This differentiation into two kinds of chromatin, one of which (basichromatin) forms chromosomes and the other (oxychromatin) does not, occurs in the early prophase; in the preceding rest stages all the chromatin, both reticulum and spherules, stains alike, figs. 45–55 and 61–64 and 69–70. In the first and second cleavages the oxychromatin granules are scattered through the whole of the nucleus and most of them dissolve *in situ*, figs. 53, 54, though some of them become attached to the mantle fibres of the spindle, fig. 55, and text figs. XVII and XVIII, where they are either transformed into spindle fibres or are dissolved, exactly as in the prophase of the first maturation. These dissolving granules sometimes remain hollow and in this case their morphology sufficiently identifies them with the chromatin spherules of preceding stages, figs. 49–52; in other eggs the dissolving granules become solid and gradually grow smaller and smaller until they disappear in an almost homogeneous nuclear sap, figs. 53 and 63. In some of the cleavages, particularly the second and third, I have observed that the basichromatin, in the form of a densely staining reticulum occupies that portion of the nucleus lying nearest the centrosome ("Pol" of Rabl '85), while the oxychromatin, also in the form of a reticulum, occupies the opposite half of the nucleus ("Gegenpol" of Rabl), figs. 62

[1] These hollow spherules with clear center and dark periphery directly reverse that common staining phenomenon, such as is characteristic of yolk spheres, where the periphery becomes clear, on destaining, and the center remains dark. They have also been figured by Korschelt ('95) in *Ophryotrocha* and by Coe ('99) in *Cerebratulus* in the prophase of the first cleavage.

and 70. These nuclei are very similar to those figured and described by Calkins ('98) in *Noctiluca* and by R. Hertwig ('99) in *Actinosphærium*, where the basichromatin is aggregated at one pole ("Hauptpol") and the oxychromatin at the other ("Gegenpol").[1]

In all cases the oxychromatin granules or reticulum completely disappear as such, though this may not happen until after the spindle is well formed, e. g., fig. 55. Wilson ('95) maintains that a portion of the chromatin (oxychromatin) is transformed into linin in *Toxopneustes*, and Griffin ('99) holds the same view as to *Thalassema*; see also Lillie (1901, p. 250). I have no doubt that this is the case also in *Crepidula*, where many of the oxychromatin granules are arranged on the linin fibres and are here dissolved and apparently transformed into the substance of the fibres (see text figs. XVII, XVIII). The further history of the achromatic substances will be followed under the head of the mitotic spindle and spheres.

The basichromatin is transformed into chromosomes in the manner already indicated (p. 36). In no nucleus in *Crepidula* have I ever been able to find a single continuous spireme thread. The chromosomes are formed by the union into a linear series of the chromatin spherules or from portions of the chromatin reticulum, but from the first there is a large number of these segments, though I cannot determine whether the number is the same as the final number of chromosomes. Perhaps this method of formation of chromosomes without a preceding spireme is to be looked upon as a modification due to a precocious segmentation of the spireme.

In the early prophase of several cleavages, particularly the first division of the first quartette, the chromatin is aggregated into a dense mass at the center of the nucleus, leaving a peripheral zone inside the nuclear membrane which contains no chromatin, text fig. XXIX. Such nuclei resemble in appearance the "synapsis" stages (Moore, Montgomery) of spermatogenesis. This condition is the result of the aggregation of the chromosomes, a phenomenon which occurs in every prophase, while the resemblance to the synapsis is due merely to the persistence of the nuclear membrane for an unusually long time.

c. Separation of Chromosomes and Formation of Daughter Nuclei. The chromosomes, which are at first widely scattered through the nuclear cavity, text figs. XVII and XVIII, are first drawn into the equatorial plate and then transported to the poles of the spindle in the usual manner.

The splitting of the chromosomes in the first cleavage, however, greatly resembles a heterotypic mitosis. In this division many of the chromosomes are shaped like rings, ellipses or triangles, and the parts of these figures lying in the equator grow thinner and thinner, the chromatic substance aggregating in the portions of the chromosomes turned toward the poles, until only a faint linin thread is left completing the otherwise open rings or triangles, fig. 56. I am not sure that this type of division of the chromosomes in the first cleavage occurs in all eggs, since I have found it in only a few cases and have been unable to find it in others of apparently the same stage (cf. figs. 56 and 57).

[1] Montgomery (1900) has rendered these names into the convenient English terms "central pole" and "distal pole," which terms I shall adopt in this paper.

The separation of the chromosomes coincides in point of time with the flow of the interfilar substance of the spindle to the spheres. The chromosomes move toward the poles until they come into contact with the spheres and even spread around them to a certain extent, figs. 59, 66, 67. Such a fact is irreconcilable with the theory that the chromosomes are moved solely by the contraction of the spindle fibres, as Wilson ('95) and Griffin ('99) have pointed out, and suggests that the movements of both interfilar substance and of chromosomes may be due to the chemotropic attraction of spheres and centrosomes, as Strasburger ('93) maintains.

When the chromosomes have reached the borders of the sphere at the end of the spindle they do not enter into the sphere but spread somewhat over its surface figs. 59, 66, 67. In this position the chromosomes are rapidly transformed into vesicles, which grow larger and larger. These vesicles then fuse together and the nucleus becomes an apparently single vesicle, though divided by a partition wall as described above (p. 34). A reticulum of chromatin is then formed within the daughter nuclei, which probably arises from the walls of the chromosomal vesicles, and on each side of the partition wall there appears a single nucleolus, fig. 60. While these chromosomal vesicles are in contact with the sphere, the latter frequently becomes pear-shaped with the pointed extremity toward the chromosomes, fig. 67. In all cases the daughter nuclei have processes which extend partially around and even into the spheres, figs. 60, 81. Gradually, however, the processes disappear as the daughter nuclei increase in size and the latter finally become rounded on the side next the spheres, figs. 61 and 68. The significance of these processes of the nucleus which project into the spheres is not far to seek. The daughter nuclei are at this stage increasing their achromatic substance at a great rate, and the form of these nuclei at once suggests that this substance is absorbed in large part from the spheres. The nuclei of the growing egg cells of *Dytiscus*, as described by Korschelt ('89), are similar in form, and perhaps in function, to this stage of these cleavage nuclei.

Lillie ('99) has observed that just before the "inner sphere" begins to expand, after the second maturation division in *Unio*, it is three-quarters surrounded by the chromosomes, and he suggests that there may be at this time a diffusion of chromatin into the sphere, the interior of which stains more darkly than before. According to my interpretation of the similar phenomenon in *Crepidula*, the chromosomes are at this time absorbing substances from the spheres; not until much later does the "inner sphere" or centrosome again stain more deeply.

During this rapid growth of the daughter nuclei the spheres decrease somewhat in size (cf. figs. 60 and 61, also 67 and 68), in spite of the fact that at this time sphere substance is collecting into the spheres from the astral radiations so that the decrease in the size of the spheres is not so great as it would otherwise be.

The chromatin reticulum which is formed in the daughter nuclei gives place in the next prophase to chromatic granules connected together by linin threads, figs. 61, 62, 70.

In early stages of the prophase, when the centrosomes are just moving into

position at the poles of the nuclei, the latter frequently put out one or more short blunt processes, text figs. VII, VIII, XII. These processes contain chromatin and sometimes dark masses which look like nucleoli. Unlike the nuclear processes of the anaphase, described above, these are usually found on the side of the nucleus away from the centrosome and nearest the mid-body. It is probable that they are withdrawn into the nucleus before the nuclear membrane is dissolved. Their significance is unknown.

This completes the account of the cycle of changes which the nucleus undergoes from one prophase of the cleavage to the next. With the exception of certain minor details, as has been pointed out, each cleavage is like every other in the matter of these nuclear changes. Apart from the equal division and distribution of the chromosomes in each mitosis, the most obvious and striking fact in this nuclear cycle is the escape of so large a part of the nuclear constituents into the cell body during mitosis and the reabsorption of a part of these by the daughter nuclei.

2. Centrosomes and Central Spindles.—*a. Centrosomes.*—The origin of the centrosomes for the first cleavage has already been described in detail (pp. 25–30). These centrosomes are at first minute granules, quite independent of each other. A few fibres are inserted in them and radiate for a short distance into the cytoplasm. Some of these fibres grow toward the nucleus and form a cone or half spindle (figs. 48, 53), while others grow between the two centrosomes and unite them, thus forming a "central spindle" in the manner observed by Hermann ('91), Drüner ('94) and MacFarland ('97). From the time the central spindle appears, the history of the centrosomes of the first cleavage is almost identically like that in the other cleavages so that the following description, unless otherwise specified, applies to any and all of the cleavages.

The minute centrosomes of the prophase (figs. 52, 53, 54, 63, 70, text fig. IV, *a* and *b*) become much larger in the metaphase (figs. 57, 65, 72, 76, text fig. IV, *c* and *d*) and stain less deeply at the center. In the anaphase (figs. 58, 59, 66) the centrosomes continue to enlarge, the periphery alone staining with haematoxylin while the central area takes the plasma stain. Finally in the late anaphase and in the telophase the centrosomes become relatively enormous spheres (figs. 60, 67, 73, text fig. IV, *e* and *f*), frequently 6 to 8 μ in diameter. The peripheral layer or centrosomal membrane grows thinner and thinner until it reaches such a degree of tenuity as to be scarcely visible, ultimately breaking up into granules (figs. 68, 69, 73, 74). In all these respects the metamorphoses of the centrosomes throughout the cleavage are the same as in the maturation divisions.

The central area of the enlarged centrosome is at first apparently homogeneous (figs. 58, 66), but gradually minute granules begin to appear within it and then extremely delicate threads connecting them into a reticulum (figs. 59, 60, 66, 67, 73, text fig. IV). Sometimes one sees, as in figs. 60, 61, 62, one or two granules within the centrosome which are slightly larger than the others; but during the telophase all of these granules are extremely minute and stain very faintly with plasma stains. Gradually they grow larger until they fill the entire centrosome and

their affinity for nuclear stains increases until in the resting stage of certain cleavage cells, these centrosomes look like small nuclei filled with a mass of minute chromatin granules, figs. 61, 69, 76, text fig. IV, *f*. In other cleavage cells these centrosomes with their contained granules remain much less conspicuous. Of all the early cleavage they are most plainly visible in the macromeres just before the formation of the first and second quartettes, figs. 69, 74, 75. The cause of this difference in the appearance of the centrosomes in different cells depends largely upon their size and affinity for stains. The size of the centrosome is always proportional to that of the cell in which it lies; its affinity for stains, during the resting period, increases as it approaches the free surface of the cell, so that although it may stain faintly when a short distance from the surface (figs. 61, 62, 68) it stains deeply when in contact with it (figs. 69, 74). Upon these two factors then depends the relative conspicuousness of the centrosome during the resting period.

In all the cleavages which I have studied, with the exception of the first, the new centrosomes, and probably also the central spindles, arise within the mother centrosome, as in the case of the second maturation spindle. The origin of centrosomes for the second cleavage is shown in figs. 62 and 63, though the origin of the central spindle could not be clearly made out in this case. The origin of centrosomes and spindles for the third cleavage is shown in fig. 70, while those for later cleavages are shown in figs. 74, 75 and 76. In all these cases the centrosomes appear as slightly enlarged granules within the old centrosome. These granules stand at some distance from each other, and in no case in the cleavage have I seen the division of a single granule to form these two; they are, however, connected by the reticulum of threads and granules which fills the mother centrosome, and when the time arrives for the formation of a new mitotic figure the mother centrosome elongates, becoming slightly elliptical in outline, the daughter centrosomes, as two enlarged granules, lie at the extremities of this ellipse, and the reticulum which fills the mother centrosome is drawn out into an irregular spindle shaped body composed of threads and granules, figs. 70, 74, 75, text fig. IV, *g*, *h*, *i*. This elongation continues and the spindle shaped body becomes the central spindle (fig. 76), which in this case consists, not of straight fibres running from pole to pole, but of irregular and anastomosing fibres with granules at their nodes. The daughter centrosomes soon become surrounded by a little area free from granules, which is due to a halo of radiating fibres, so fine that few of them can be seen at this stage. This is the first appearance of the sphere ("couche corticale") and it also arises, at least in certain cleavages, within the mother centrosome, figs. 70 and 76, the membrane of which may still persist at this stage.

At a slightly later stage these radiating fibres become very evident, and with the formation of the cones or half spindles, as described at the beginning of this account of the centrosome, we have the completion of the cycle of changes undergone by the centrosome from one prophase to the next. In a word, the most important features of this cycle are (1) the great increase in size of the centrosome and its transformation into a sphere filled with a reticulum of fibres and granules, and

(2) the origin of the new centrosomes and central spindle from this reticulum; in some cases at least the cortical zone also arises within the old centrosome, so that the entire initial spindle of one cell generation arises within the centrosome of the preceding generation.

b. Central Spindles. In the case of the first cleavage the central spindle is formed after the centrosomes have taken their definitive positions at the poles of the germ nuclei, figs. 55 and 56. It first appears as a few fibres running from centrosome to centrosome in the line of contact between the egg and sperm nuclei. These fibres run independently from pole to pole and do not branch and show cross anastomoses with one another, so far as I have been able to observe; there are no varicosities or granules on them, as is the case in the later cleavages. The central spindles for the second cleavage are shown in surface view in fig. 82 and text fig. VIII, running from centrosome to centrosome over the surface of the nuclei and in the groove between the nuclear halves. In a section of a somewhat earlier stage (fig. 63), I have been unable to detect the fibres of the central spindle, though there is a clear area free from granules lying immediately over the nucleus and between the centrosomes in the position of the central spindle.

In the later cleavages the origin of the central spindle within the mother centrosomes can be plainly observed figs. 70, 74, 75, 76. The central spindle is in these cases a long drawn out reticulum with granules at its nodes. These granules gradually disappear as the spindle elongates and their substance is evidently transformed into the central spindle fibres.

In *Crepidula*, then, there appear to be two methods of origin of the central spindle: in the first cleavage the spindle arises in the cytoplasm between two independent centrosomes; in all the other cleavages the centrosomes and central spindle arises as a unit structure within the mother centrosome; in the former case the fibres arise *de novo* between the centrosomes, in the later they arise as a centrodesmus (second maturation) or from the centrosomal reticulum (later cleavages).

3. Polar Rays and Spindle Fibres.—When first visible the polar rays are extremely short and delicate fibres and their presence is to be recognized rather by the clear area ("cortical zone") surrounding the centrosome than by the recognition of individual fibres, figs. 70, 76. Soon these fibres become larger and longer and are plainly visible, figs. 52, 53, 63. Those directed toward the nucleus become stouter and more numerous than the others, and the nuclear membrane is frequently indented where they come into contact with it, figs. 53, 54, 71. In some cases, however, the nuclear membrane is not indented, but is drawn out into a cone, the apex of which lies near the centrosome. Whether the membrane is invaginated or evaginated, there is in both cases an escape of achromatic nuclear substance at the poles, and it is due to this substance that the extra-nuclear fibres grow stouter and become covered with oxychromatin granules, text figs. XVII–XIX. In the first maturation division, not only the fibres of the extra-nuclear spindle, but also all the polar fibres are studded with these granules; in the cleavage, however, I have not observed them on the polar fibres. In early

prophases the fibres of the extra-nuclear spindles are directly continuous with the linin threads of the nucleus, which they closely resemble in every respect, text figs. XVII and XVIII. Like the linin they branch and anastomose and are studded with oxychromatin granules. This resemblance is so striking that I cannot doubt that the fibres of the extra-nuclear spindles are really derived from the achromatic substance of the nucleus.

As in the maturation, so also in the cleavage there is an interfilar substance which fills the spaces between the fibres and which constitutes the greater part of the bulk of the amphiaster. This interfilar substance is probably derived in part from the hyaloplasm of the cell body and in part from nuclear sap containing dissolved oxychromatin.

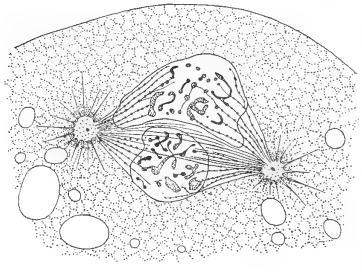

FIG. XVII.—Prophase of first cleavage of *Crepidula*.

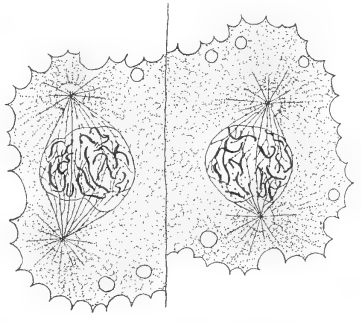

FIG. XVIII.—Prophase of second cleavage of *Crepidula*.

Throughout the metaphase the spindle-fibres are to a great extent concealed by this interfilar substance which fills in the whole space between them. In strongly destained specimens, however, the fibres can always be seen in the spindle. After the metaphase, however, no fibres can be seen crossing the dark zone which now surrounds the centrosome; both polar fibres and spindle-fibres appear to stop at the boundary of this cortical zone, or rather sphere. In the anaphase the structure of the sphere is such that one may be quite sure that neither polar nor spindle-fibres run through it, figs. 58, 59, 60, 66, 67, 68. In both metaphase and anaphase the polar fibres are not always centered on the centrosome, and if they were continued in a straight line through the sphere some of them would not touch the centrosome at all, figs. 57, 58, 60, 65, 67.

Just before the chromosomes reach the boundary of the spheres the mitotic figure is cylindrical in shape and consists almost entirely of interzonal filaments, figs. 58, 66. As soon as the chromosomes have reached the spheres and are transformed into vesicles, figs. 59, 67, the spindle again becomes wider in the middle than at the ends and contains many fibres which do not reach from pole to pole.

The spindle greatly increases in length from the prophase to the telophase. R. Hertwig ('99) has observed that in *Actinosphærium* the spindle more than doubles in length during this period, and in *Crepidula* the lengthening is nearly as great. The shape of the spindle varies greatly from prophase to telophase, being largest at the equator in the prophase and smallest in the telophase.

Mid-body.—When the new cell-wall is formed the spindle is constricted in the middle and a very remarkable mid-body (*Zwischenkörper*) is formed. This mid-body is elliptical in outline, and is surrounded by a dark area from which radiations proceed in all directions; into this dark area the cell-membrane and the two halves of the spindle enter, fig. 60. This mid-body is for all the world like a centrosome with its surrounding sphere and aster, and recalls Watase's ('93) comparison of the mid-body to an intercellular centrosome. This apparent resemblance is still further supported by the fact that the mid-body in this case becomes a hollow sphere before it finally disappears, fig. 61, just as the centrosome does.

The mid-body is surrounded by a darkly staining substance which resembles the sphere substance. This recalls Moore's ('93) observations on the larval Salamander, where he finds a mass of archoplasm on each side of the mid-body, also Kostanecki's ('92) statement that the mid-body is formed from granules of the sphere (archoplasm). Kostanecki ('97) has observed a mid-body in *Physa* consisting of a ring around the central spindle-fibres, from which radiations proceed. In some cases this ring divides through the middle into two rings. A similar ring is called by Heidenhain "*Zellnabel*." Moore and Meves have seen mid-bodies connected with the centrosomes around the nucleus, as is plainly the case in the third cleavage of *Crepidula* (see fig. 73).

The cell-membrane adjoining the mid-body is thicker and more protoplasmic than at the periphery, and is in process of formation at this place. The mid-body persists through the whole of the resting period and until the prophase of the next succeeding division when it gradually disappears. As long as it is present there can be no doubt as to protoplasmic continuity between the daughter cells.

4. Spheres.—Before the nuclear membrane is indented, the centrosomes are surrounded by a clear area consisting of a halo of radiating fibres, figs. 63, 70, 76. This condition may exist even within the mother centrosome (see fig. 70). This clear area is the first appearance of what I shall call the sphere ("outer sphere" of Lillie, "couche corticale" of Van der Stricht). When the nuclear membrane is dissolved at the poles substances escape from the nucleus into this area surrounding the centrosomes. At the same time hyaloplasm from the cell body is probably drawn in through the astral rays into the same area. There is thus a commingling of hyaloplasm and chromatic nuclear sap which constitutes the interfilar substance of the aster. There is at this stage no clearly marked sphere, since the central area of the aster is in no way delimited from the surrounding radiations.

In middle stages of mitosis it is difficult, even in thoroughly destained specimens, to trace the polar rays and spindle-fibres through the interfilar substance to the centrosome. In the anaphase the interfilar substance of spindle and aster

collects into the central area surrounding the centrosomes, and this area, thus delimited from the surrounding plasm, is the sphere; at the same time the spindle-fibres again become plainly visible while a reticular or alveolar structure appears within the spheres, fig. 58.

In the late anaphase the spheres become much larger and are bounded by a layer of microsomes from which fibers radiate. The interior of the spheres is composed of a fine reticulum with nodal thickenings, and the whole sphere stains much less densely than in earlier stages. Finally in the telophase the spheres reach their greatest size and become filled with granules, the reticulum being scarcely visible, or disappearing altogether (figs. 61, 68, 73).

During the whole of the resting period the spheres persist, usually pressed close to the cell-membrane, and as long as the centrosomes remain in them they preserve a regular form (figs. 68, 69, 73, 74, 76). They are composed of coarse granules, which stain deeply with plasma stains, and they are sharply bounded by one or more layers of microsomes. As soon as the daughter centrosomes and central spindle arise from the mother centrosome, they migrate out of the sphere and the latter at once begins to lose its regular form. It becomes ragged in outline and is finally flattened out to a thin layer of densely staining granules immediately under the cell-membrane (figs. 63, 65, 70, 71, 72, 73, 74, 76).[1]

These granules, the remains of former spheres, can frequently be recognized through two generations of cleavage cells; *e. g.*, the spheres which appear in the second cleavage (figs. 68–72) can still be recognized after the completion of the third cleavage, located in the first quartette of micromeres (figs. 73 and 74, see also figures of entire eggs in Plates V and VI). From the time when the daughter centrosomes issue from the spheres the latter are degenerating structures, and although their remains may persist for a surprisingly long time they ultimately disintegrate and are apparently dissolved in the cytoplasm.

To sum up the history of the spheres: we find that they arise around the centrosomes at a very early period in the mitosis, in some cases within the mother centrosome. With the disappearance of the nuclear membrane at the poles of the spindle they are invaded by an interfilar substance; they have no clearly marked boundary. In the anaphase and telophase the spheres greatly enlarge, but their growth is always proportional to the size of the cell in which they are found. They are largest in the anaphase just before the chromosomal vesicles begin to form and they probably contribute to the growth of the daughter nuclei. At first they have a delicate radiating structure, this gives place to a homogeneous condition, and this to an alveolar or reticular one; finally, in the rest stage they are granular. Their fragments persist long after the daughter centrosomes have moved out of them, and they ultimately dissolve and disappear in the cytoplasm.

[1] In surface views of entire eggs the sphere may seem larger in the resting stage or early prophase than in the telophase, *e. g.*, figs. 81 and 82, 86 and 87, etc.; this is due, as sections show, to a flattening of the sphere against the cell-membrane and a spreading of the sphere substance through the influence of the astral rays, and not to an actual increase in its volume (figs. 71, 72, 76).

IV. General Considerations and Comparisons.

I propose to give in this section a brief synopsis of the changes which the nucleus, centrosome and sphere undergo during the whole cycle of division in the mollusks which I have studied; to compare these observations with closely related ones in other animals and to indicate the general conclusions to which these observations lead.

1. The Nucleus during the Cycle of Division.—The history of the nuclear changes during the cycle of division may be summarized as follows: (1) The chromosomes, consisting of chromatin inclosed in a linin sheath, divide and move to the poles of the spindle where they partially surround the spheres. (2) Here they become vesicular, the interior of the vesicle becoming achromatic, though frequently containing a nucleolus-like body, while the wall remains chromatic. (3) These vesicles continue to enlarge and then unite into the "resting nucleus"; the nuclear membrane is composed of the outermost walls of the vesicles, while the inner walls stretch through the nucleus as chromatic partitions; the chromosomal vesicles from the egg and sperm nuclei remain distinct longer than those from the same nucleus. (4) The chromatin of these inner alveolar walls then aggregates into threads, giving rise to a "chromatic reticulum," though the linin still preserves, for a time at least, the alveolar structure. (5) The chromatin of these threads then aggregates into spherules, which are connected together by linin threads; these spherules vary in size, and at first all are solid and stain alike. (6) They then become hollow and are differentiated into oxy- and basi-chromatin. (7) In the first maturation each of the basichromatin spherules, or bodies, grows into an individual chromosome; in the cleavage the basichromatin spherules unite into several linear series, thus forming a segmented spireme. (8) The oxychromatin spherules grow smaller and some are dissolved in the nuclear sap while others are arranged in series on the linin threads into which they are transformed; these threads with attached spherules form the spindle fibres. (9) During the differentiation of the chromatin the nucleus swells in size and the membrane becomes less chromatic, while the nuclear sap becomes more so; the nuclear membrane then dissolves at points opposite the centrosomes and linin, oxychromatin and nuclear sap here escape. (10) The spindle, which at first fills the entire nuclear cavity, then grows longer and slenderer and contains an interfilar substance; the nuclear membrane entirely disappears; the equatorial plate stage is then reached and the cycle is complete. In a word, the daughter chromosomes absorb achromatic substances, and unite to form the nucleus, within which the chromosomes and spindle of the next division arise, while nuclear sap and dissolved chromatin escape into the aster and cell body.

Taking up now in more detail some of the individual steps in this cycle:

(*a*) *Formation of Chromosomal Vesicles.—Growth of Daughter Nuclei.*—When the chromosomes have reached the ends of the spindle, and in some abnormal cases even before this (see text fig. IX), they begin to absorb achromatic material and to swell into spherical vesicles. Such vesicles are found generally, if not uni-

versally, in the early divisions of ova, though they are not usually found in other mitoses. What is the cause of this difference? It occurs to me that it may be due to differences in the size and in the rapidity of division of blastomeres as compared with tissue cells.[1] The following observations favor this view: — The chromosomal vesicles are proportional in size to the size of the cell (quantity of cytoplasm) in which they lie. The daughter chromosomes which go to the two poles of the spindle are always equal in size however unequal the cell division may be, until the time when the daughter cells are separated by the new cell wall. Immediately after this separation a difference appears in the size of the vesicles in the two cells, if the division was unequal, the larger cell containing large chromosomal vesicles while in the smaller cell they remain small or do not show the vesicular structure at all.

The chromosomes which go into the polar bodies do not appear vesicular at any stage, though after the division of the first polar body they fuse into a single nucleus in each cell which contains very little achromatic material. The smallest cells in the early stages of cleavage are the "trochoblasts" (fig. 97, $1a^2$–$1d^2$); these cells do not again divide for a very long period, and in them the chromosomal vesicles are at first very small. Chromosomal vesicles appear in the anaphase of all the other cleavages, but as the cleavage advances and the blastomeres grow smaller these vesicles become less and less apparent.

From these observations I conclude that in large cells where divisions succeed one another at short intervals the chromosomes begin the growth characteristic of the daughter nuclei, *i. e.*, the absorption of substances from the cell body, before they have fused together, whereas in small cells or cells which divide only at long intervals the chromosomes fuse before the absorption of achromatic material begins.

After the fusion of the chromosomal vesicles to form the daughter nuclei, the latter continue to absorb achromatic material, growing larger and larger, until the prophase of the next division. A part at least of the achromatic material absorbed is derived from the sphere which in turn contains interfilar substance of the spindle and aster. This recalls the conclusions of O. Hertwig ('75), in which he points out that in the formation of the daughter nucleus the chromosomes absorb "*Kernsaft*" and become vesicular, the process being the reverse of what occurs in the beginning of division, when "*Kernsaft*" is set free into the cell body. A similar view was held by Bütschli ('76).

In the growth of the nucleus the nuclear membrane has the properties of a semi-permiable membrane, *i. e.*, substances pass readily through the membrane in one direction, but not in the other. Reinke (1900) has suggested that the nuclear ground substance is a diosmotic material, which, by taking up substances from the cell, produces a substance of higher osmotic pressure. When, on the other hand, the nuclear membrane dissolves and the ground substance of the nucleus mingles with the fluid substance of the cell, the peripheral layer of the latter assumes the

[1] Flemming ('92) formerly held that all chromosomal vesicles were artifacts. Now that they have been found, however, in so large a number of ova, prepared by the best modern methods, such an idea cannot be maintained.

role of a semi-permeable membrane and thereby the swelling of the dividing cell is produced which Reinke calls "mitotic pressure." The manner of growth of the nucleus, its turgescence and the infolding of its membrane in the prophase preclude the idea that the nuclear membrane is full of pores as held by Carnoy, Watase, and formerly by Reinke, and indicate that the growth of the nucleus is a phenomenon of diosmosis.

However unequal the division of the cell body may be the daughter nuclei are at first entirely equal, but the subsequent growth of the nucleus is proportional to the quantity of cytoplasm in which it lies; this is shown not only in the cleavage of the egg, but also in the formation of the polar bodies. The nuclei of the polar bodies rarely become vesicular but remain chromatic throughout. The fact that the size of the nucleus is proportional to the quantity of the cytoplasm in which it lies indicates that the achromatic substance absorbed by the nucleus is also proportional in quantity to the volume of the cytoplasm.

It sometimes happens, especially in eggs in which more than the normal number of centrosomes and asters are present, that some or all of the chromosomal vesicles do not fuse, but remain distinct through the whole of the resting period. In such cases each of the vesicles behaves like a miniature nucleus, absorbing achromatic material and forming a network of chromatin either within the vesicle or on its walls, In this growth and differentiation the vesicles keep pace, step by step, with the normal nucleus, so that one must regard the resting nucleus as virtually composed of vesicles, though their union may be so intimate as to hide this structure. The resting nucleus is not, therefore, a single structure any more than is the equatorial plate. It is composed of units, each of which, so far as known, has the properties of the entire nucleus, and the union of these vesicles into a single one may be considered as a secondary character. It is altogether probable that the chromosomes, and hence the chromosomal vesicles, preserve their identity throughout the resting period, and I venture the suggestion that the daughter chromosomes will be found to arise within the chromosomal vesicles, as the daughter centrosomes, or centrioles, arise within the mother structures.

(*b*) *Chromatic Differentiation; Solution of Oxychromatin and Nuclear Membrane.*—In the early prophase of each division in the mollusks which I have studied, the chromatin becomes sharply differentiated into oxy- and basi-chromatin (Heidenhain). This differentiation occurs before the solution of the nuclear membrane, but at a time when the nucleus is growing rapidly in size and is therefore actively absorbing substances from without. This suggests that the rapid absorption of cell substance and the differentiation of the chromatin are associated, but whether this absorption is the cause or the result of the chromatic differentiation, I am unable to determine.

The solution of the nucleoli usually precedes that of the oxychromatin spherules and the nuclear membrane, but in the case of the first maturation the enormous nucleolus is thrown out into the cytoplasm before it is completely dissolved. Many oxychromatin granules are not dissolved in the nuclear sap,

but contribute directly to the formation of the linin threads and spindle fibres, as Wilson ('95) and Griffin ('99) have found to be the case in *Toxopneustes* and in *Thalassema*. This is especially the case in all small nuclei, whereas the larger the nucleus the greater the quantity of oxychromatin which dissolves and passes into the cytoplasm.

The solution of the nuclear membrane goes on coincidently with the solution of the oxychromatin, so that it seems probable that the causes of the two are the same. Before its solution the membrane changes its staining qualities, becoming more and more plasmatic in reaction. The membrane is in all cases first dissolved at points opposite the centrosomes. In this process the membrane undergoes one of two modifications: either (1) it is drawn out toward the centrosomes into a cone-like figure, or (2) it is indented opposite the centrosomes. Both of these methods may coexist in the same animal, though one or the other is usually predominant. Among mollusks of all classes, the membrane is usually indented. The difference between these two methods is not great, depending upon the time at which the membrane is dissolved and upon the rate of outflow of nuclear substance; if the membrane is thin and dissolves early a cone is formed; if it dissolves slowly and only after a considerable quantity of nuclear substance has escaped, it becomes indented. The strength of the nuclear membrane in *Crepidula* is shown not only by the degree of indentation which it suffers before it is completely dissolved at the poles, but also by its long persistence in the equator of the nucleus (see figs. 84 and 88). Even when the membrane persists for a long time and becomes deeply indented at the poles it need not be supposed that pressure is brought to bear by the polar fibres or by other means upon the membrane; on the contrary, the indentation is chiefly or entirely due to the escape of nuclear sap and the consequent collapse of the nuclear wall.

The infolding or outfolding of the nuclear membrane at points opposite the centrosomes is a very common phenomenon among all classes of animals. It would be useless to attempt to summarize all the observations on this point, and I shall refer to only two recent works which touch upon this subject:—

Montgomery ('98) has observed a cone-shaped protrusion of the nuclear membrane opposite each centrosome in *Pentatoma*. These cones contain a dark substance which he believes to be of nucleolar origin.

Fischer ('99) interprets the openings at the poles of the nucleus as due to a greater growth of the nucleus at these points. The fact, however, that it first occurs opposite the centrosomes and in connection with the formation of the half spindles, indicates that the opening in the nuclear membrane is due rather to the solvent action of some substance which diffuses to or from the centrosomes.

(*c*) *Escape of Nuclear Substances; Aster and Spindle Formation.*—It may be considered certain that the infolding (or outfolding) of the nuclear membrane at points opposite the centrosomes is due to an outflow of nuclear substance at these points. This is conclusively shown by the fact that the linin reticulum, with its attached chromatin granules, here extends outside the nuclear wall nearly to the

centrosome and forms the extra-nuclear portion of the spindle. The aster also stains more deeply after this outflow than before and very like the chromatic nuclear sap.

The shape of the spindle depends in part upon the degree and stage of this nuclear outflow. The spindle is at first as wide at the equator as the entire mother nucleus, but as the flow of nuclear substance toward the poles continues it grows longer and slenderer, the centrosomes at the same time moving farther and farther apart, until in the late anaphase almost the whole of the interfilar substance has moved out of the spindles into the spheres.

Whether or not the spindle fibres and linin threads exist as such in the living cell or are artefacts must still be left an open question. It can scarcely be doubted, however, that they do represent substances which are different from the surrounding materials of the cell, and this is after all the important thing. That the spindle-fibres and especially the connective fibres sometimes show considerable elasticity and rigidity has been pointed out repeatedly by those who hold that the centrosomes are pushed apart by their activity. Nowhere is this better shown than in the first maturation of *Crepidula*, where in the shortening of the spindle the fibres are bent and kinked and the chromosomes at the outer pole are pushed clear through the polar body into contact with the opposite cell wall. In spite of this, however, it seems to me very questionable whether the spindle fibres are anything other than a fluid more viscid than the surrounding cell substance.

I agree with those authors (Bütschli, Fischer, Rhumbler, Wilson), who hold that the astral rays represent diffusion streams in the cytoplasm, rather than a stable system of fibres. There are certain evidences that the astral rays are composed in the main of cytoplasmic material, principally hyaloplasm or interalveolar substance; chief among these is the fact that the aster is always proportional in size to the extent of the cytoplasmic area which comes within its influence, a fact which Wilson ('96) emphasized and which I also ('94) pointed out and have since had abundant opportunity to verify. But while the aster and astral rays are in the main composed of hyaloplasm it is probable that in normal mitoses certain nuclear substances enter into their formation. In the mollusks which I have studied there can be no doubt that certain achromatic substances from the nucleus and spindle flow into the aster and at the same time the central area of the aster as well as its rays stain more deeply than the hyaloplasm throughout the cell body. Whether there may not be a centrifugal movement of escaped nuclear substance along the astral rays as well as a centripetal movement of the hyaloplasm must be left an open question.

In this connection I must refer to one of the first observations ever made on indirect nuclear division,—that of Auerbach ('74) on the living eggs of certain nematodes. He observed the double suns (asters) with their connecting stalk (spindle) and supposed that they were formed by the collapse of the nucleus and the passing out of nuclear sap into the cytoplasm, "the astral radiations being merely the expression of the paths along which fine streams of nuclear sap pass out into the protoplasm." Bütschli ('76) also observed the passage of nuclear sap

into the cytoplasm during nuclear division; in *Cucullanus* and *Nephelis* this loss of nuclear fluid may amount to as much as two-thirds of the volume of the unaltered nucleus. Bütschli held that this fluid escaped at the two poles of the nucleus and accumulated in the central areas (asters), from which it radiated into the cell body. Further Bütschli observed that the more a daughter nucleus grows, the more the central area of the neighboring radial system diminishes, whence he inferred that the latter furnishes material for the growth of the former. (See Mark, '81, p. 321.)

In 1892 Bütschli reversed his former view as to aster formation, holding that it is due to a flowing of plasma into the spheres or centrosomes and not from them. He supposed that the centrosomes attracted substances dissolved in the enchylemma as a hygroscopic substance attracts water, and that the diffusion movements thus produced cause the astral radiations. Although Bütschli in his 1892 work, and since in 1898 and 1900, maintains that the astral radiations are due to an attraction exerted by the centrosome, he expressly stated in the first mentioned work that it is unimportant whether the diffusion streams move in one direction or the other (*i. e.*, centrifugally or centripetally).

I fully agree with Bütschli that the astral rays are the expression of diffusion streams. In the process of diffusion the commingling substances may move in opposite directions at the same time, and it is quite possible that the balance of flow between the centripetal and the centrifugal diffusion movements may lead to a centrifugal flow at one time and to a centripetal flow at another.

Rhumbler ('96, '99) has also developed an elaborate theory of aster and spindle formation which is based in the main upon this view of Bütschli's. He holds that the astral rays are reducible to tension on the alveolar radii; this tension being due to the fact that the centrosomes, and later the nucleus, take up fluids from the surrounding plasm. He also holds that the two spheres exert a pull on the nucleus which leads to the formation of the spindle and to the escape of nuclear sap into the equatorial plane of the cell, where the division wall will form.

I accept Rhumbler's views as to the flow of cell and nuclear substances toward the centrosome, but cannot agree with him that the nuclear sap escapes largely or entirely in the equatorial plane. Much of the nuclear sap as well as the oxychromatin and linin escapes at the poles of the nucleus and, although nuclear contents escape into the cytoplasm in all directions when the nuclear membrane is completely dissolved, there is no evidence in the cases which I have studied that this has to do with the formation of the division wall.

Fischer ('99) holds that the spheres of animal eggs are to be explained as substances escaped from the nucleus, and he suggests that the astral rays may be normally formed by the diffusion of substances from the nucleus into the cytoplasm and the production there of non-soluble substances. He considers that such radiations are not persistent structures, but that they may appear and disappear repeatedly during the course of a single division. I agree with Fischer that there is an escape of nuclear substance at the poles of the nuclei, and that the astral rays

represent diffusion streams through the cell body, but I am not sure that I understand him when he says that the rays are composed of non-soluble substance, since they certainly disappear (as he also maintains) either by dissolving in the cytoplasm or by being absorbed into the sphere.

Meves ('99) criticises Fischer's views on aster formation by saying that such rays as Fischer describes could not grow interstitially, as normal rays and spindle fibres are known to do; sometimes also extensive rays appear in the anaphase, long after the mingling of nuclear and cytoplasmic substances. If, however, these rays be considered as diffusion streams to or from the centrosome, in the sense of Bütschli, these criticisms lose most, if not all, of their force. Finally, that the astral rays are not fixed structures stretching between the centrosome and the cell membrane, as Heidenhain and Kostanecki hold, is shown by the fact that in many mitoses the spindle is free to turn and move through the cell, and yet the astral rays show neither twisting, bending, nor distortion. This is shown especially well in the first maturation, and in the first three cleavages of *Crepidula*, where there are considerable movements of the amphiaster even after the metaphase; but in no case is there a corresponding bending of the rays, as there would be if these were fixed structures (see observations of Ziegler, Lillie, *et al.*, Part II, Sec. III).

(*d*). *Chromatic Elimination.*—In the maturation and early cleavages of the eggs which I have studied, the total amount of chromatin which is transformed into linin or dissolves and escapes into the cell body is greater than that which goes to form the chromosomes; the amount of cytoplasm in the cell is also noticibly greater after the nuclear membrane is dissolved than before.

In this connection other observations of a somewhat similar character may be recalled. Almost all persons who have studied the maturation of the egg, have commented upon the large quantity of nuclear material which is set free into the cell body during the first maturation division. In the starfish, according to Wilson ('95, p. 458), at least nine-tenths of the chromatin is thus set free. Gardiner ('98, p. 97) estimates that in the egg of *Polychærus* not more than one five-hundreth part of the chromatin which is present in the germinal vesicle goes into the chromosomes, all the rest being thrown out into the cell. Most observers agree in identifying as chromatin this nuclear material which escapes into the cell body, though in most cases it stains less deeply than the chromosomes and its subsequent dissolving shows that it must be different from the chromosomes, which never dissolve. Gardiner ('98, p. 98) argues that there must be two kinds of chromatin, the one soluble, the other not, and Griffin ('99) believes that the soluble chromatin arises as a nuclear reticulum which at first takes plasma stains and later nuclear ones.

Boveri's ('92 and '99) observations on the diminution of the chromosomes in the somatic cells of *Ascaris* may be recalled in this connection. In this case the ends of the chromosomes pass into the cytoplasm during the mitosis and there gradually undergo solution or disintegration. This case, however, differs greatly from that of *Crepidula* since it occurs only in differentiation of somatic cells, whereas in *Crepidula* the outflow of nuclear material occurs at each and every

mitosis. Häcker ('97) has described an elimination of nuclear constituents in the *Keimbahn* of *Cyclops;* in the first cleavage a large number of granules ("ektosomes"), which Häcker considers escaped nucleoli, collect around one attraction sphere but not around the other. This process is repeated in subsequent cleavages, the cells in which the ektosomes appear marking out the *Keimbahn*. Finally, in the division of the genital cells the ektosomes are found around the entire spindle figure. The elimination of the ektosomes in *Cyclops*, like the diminution of the chromosomes in *Ascaris*, differs fundamentally from the chromatic elimination in *Crepidula*, in that the latter occurs in all the cleavages irrespective of whether the blastomeres are progenitors of the germ cells or not.

In the ovarian eggs of many animals an elimination of nuclear constituents has been observed (for a list of these cases see Meves, '94, p. 149); all these cases deal with elimination during the resting period of the nucleus. On the other hand my observations mentioned above, as well as those of Wilson ('96, p. 141) on *Nereis*, Mathews ('95) on *Asterias*, Gardiner ('98) on *Polychærus*, Griffin ('99) on *Thalassema*, and many others, show that there is an escape of chromatic substance from the nucleus into the cytoplasm during the period of mitosis. In the cases just mentioned this elimination occurs during the first maturation division, and Griffin at least, affirms that it does not occur in the cleavage mitosis. In *Crepidula*, on the other hand, it occurs in every mitosis (except that of the second maturation), though it is, of course, most evident where the nucleus is large and the amount of chromatin great.

In this connection the theoretical conclusions of De Vries, Weismann and Roux, concerning the nuclear control of the cell should be recalled. De Vries holds that there is an actual migration of pangenes from the nucleus into the cell body, these pangenes giving character and direction to all cytoplasmic processes, in fact, both De Vries and Weismann assume that the entire cytoplasm is the product of the pangenes. Roux holds that the nuclei become progressively specialized during development, and that these specialized nuclei determine the character of the cytoplasm, but he does not suggest how this determination occurs. Weismann accepts and unites both the views of De Vries and those of Roux.

Judging these theories by the facts of chromatic elimination in *Crepidula* and other gasteropods, I am compelled to conclude that in all nuclei the chromatin appears the same in character, differing only in quantity; in all nuclei the chromatin is differentiated into oxychromatin and basichromatin, the latter alone forming the chromosomes, while the former is eliminated; there is no evidence of progressive differentiation of the nuclei. That these facts, however, are not conclusive against the theory of Roux is shown by the fact that in *Ascaris* there is a specialization of the somatic cells as distinguished from the germ cells; if such a specialization occurs in *Crepidula* it must begin at a much later period than in *Ascaris*. On the other hand, the fact that the eliminated chromatin is differentially distributed to the cleavage cells (see Part II, Sec. II) may be held to afford evidence of the fact that it plays some part in the differentiation of blastomeres.

But whatever the theoretical bearings of this elimination may be, there can be no doubt of the fact that in *Crepidula*, and perhaps in a large number of animals, there is a very extensive exchange of material between the nucleus and cytoplasm, and, further, that a large part of that most characteristic nuclear substance, the chromatin, passes into the cytoplasm in the form of oxychromatin during every cell cycle, while a relatively small portion is preserved for the purpose of reproducing the daughter nuclei.

There is thus in karyokinesis a rhythmical growth and collapse of the nucleus as a whole, the new nuclei arising endogenously, *i. e.*, from chromosomes, within the old. In fact, one might speak of these changes in the nucleus as a systole and diastole (Ryder, '94), by means of which an exchange of nuclear and cytoplasmic material is brought about.

The nuclear membrane appears to permit the passage of materials inward but not outward during the resting period, whereas the escape of nuclear material into the cell is brought about by the disappearance of the nuclear membrane during karyokinesis. Such a process is not universal, for in cells where karyokinesis occurs very rarely, or not at all, the interchange between cytoplasm and nucleus has been observed to take place, but the phenomena described are characteristic of mitosis in general.

2. Centrosomes and Central Spindles.—*a. Structure and Metamorphoses.*— It is evident from the history of the centrosomes of *Crepidula* that throughout the maturation and cleavages up to at least the 60-cell stage, the centrosomes are absolutely continuous from one cell generation to the next, with the possible exception of the fertilization stages. Of this fact there can be no particle of doubt. With the exception of the earliest origin of the centrosomes of the first maturation, and with some reserve as to the origin of the centrosomes of the first cleavage, I believe that I have seen every step in the origin and metamorphoses of the centrosomes up to the 12-cell stage; while in all the cleavages up to the 60-cell stage, or even later, I have observed and drawn the centrosomes at almost every stage in the cell cycle. Fortunately, this is not a very difficult thing to do, since the centrosomes are so large and distinct that even during the height of the resting period they can be seen in entire eggs, and their elongation to form the central spindles plainly observed (see Plates V, VI).

The principal features of the entire centrosomal cycle from one cell division to the next may be summarized in a single sentence: *The minute granules at the poles of the central spindle enlarge until they become hollow spheres within which new centrosomes and central spindles appear.* The individual variations characteristic of the maturation and the different cleavage stages have been mentioned in detail, and need not be reviewed here; it is sufficient to say that the history of every centrosome conforms to the above statement.

From this it is evident that the centrosomes and central spindles form a unit structure, as Heidenhain ('94), MacFarland ('96) and Boveri ('01) maintain. Only in the fertilization is this not the case, and there are few, if any, well authenticated

cases on record in which the centrosomes and central spindle of the first cleavage form a unit structure. Even in many of those cases in which there is a division of the sperm centrosome and a well-marked central spindle between the halves (*e.g.*, *Physa*, *Pleurophylidia*, *Unio*, *Cerebratulus*, *Thalassema*, *Arenicola*), this central spindle completely disappears and the definitive spindle is formed *de novo* between independent centrosomes. In view of the unit structure of centrosomes and central spindles in other divisions, this is certainly a striking phenomenon, and indicates that the centrosomes of the first cleavage are in their first appearance more independent of each other than in any subsequent cleavage. It also suggests a possible way of unifying the conflicting accounts as to the origin of the cleavage centrosomes.

(*b*). *Relation of Centrosome to Cell Body and Sphere.*—In the mollusks which I have studied, the centrosome is at all stages in its cycle sharply delimited from the surrounding cell-body and sphere. The outer zone of the mother centrosome does not disintegrate and lose its outlines until after the daughter centrosomes and spindle have appeared within it, so that in all stages of the cell cycle there is a clearly marked centrosome. In the cleavage the outlines of the centrosome are most difficult to distinguish in the anaphase (figs. 59 and 67), but even at this stage there can be no doubt of its sharp separation from the surrounding sphere. Only in the egg and sperm asters during the approach of the germ nuclei is this separation completely lost. No clearly marked sperm centrosome can be recognized at any stage, and the egg centrosome which is very evident during the anaphase of the second maturation, and which during this period undergoes a typical transformation into a hollow sphere (figs. 32–36), loses its outlines and completely disappears in the surrounding sphere before the union of the germ nuclei. This again marks a peculiarity in the centrosomes during fertilization not found in any other cell cycle.

The centrosomes and spheres grow simultaneously reaching their greatest size in the telophase or resting period when the astral radiations are smallest; the astral radiations again become prominent when the new centrosomes have moved out of the old centrosomes and sphere and are growing rapidly in size. The growth of the centrosomes and spheres is not coincident with that of the nuclei; on the contrary they are smallest when the nuclei are largest, viz., in the early prophase, and they have nearly reached their largest dimensions when the nuclei are smallest, *i. e.*, in the late anaphase before the formation of the chromosomal vesicles (*cf.* figs. 3 and 16, 27 and 34, 53 and 59, 63 and 67). This, as well as other morphological phenomena involved in the escape of achromatin at the poles of the spindle and the coincident growth of the spheres and centrosomes, together with the changes in the staining reactions of the latter, indicates that the spheres and centrosomes grow in part at the expence of substance escaped from the nucleus. That this is not a complete statement of the facts, however, is shown by all cases of unequal cleavage, in which the centrosomes and spheres at the two poles of the spindle are always equal until the constriction of the cell body begins (figs. 15, 33, 72, etc.), but immediately after this they become unequal in size, and in the end are proportional

in size to the quantity of cytoplasm in which they lie (figs. 16, 34, 73, etc.). We have already seen that the nucleus is always proportional in size to the cytoplasm in which it lies, and we are also compelled to conclude that the size of the centrosome and sphere depends ultimately upon the quantity of cytoplasm. This must be taken to indicate that both centrosome and sphere receive substance from the cytoplasm during their period of growth, and on the other hand it can plainly be seen that the remnants of the old centrosomes and spheres are slowly transformed into cytoplasm or cell membrane after the new centers have moved out of them. There is, therefore, an interchange of substance between cytoplasm and centrosome, wholly similar to that between cytoplasm and nucleus (see p. 53).

(*c*). *Relation of Centrosome to Nucleus.*—In certain cleavages the centrosomes, especially during the resting period, are very large and conspicuous, *e. g.*, in the pause preceeding the third and fourth cleavages (figs. 69, 74), they are fully six μ in diameter. They contain a reticulum of material which stains blue or black with hæmatoxylin, and on the whole they present an appearance remarkably like nuclei. In no case, however, have I seen any evidence that the centrosomes are directly derived from the nuclei, though this may possibly be the case in the origin of the centrosomes of the first maturation division; on the other hand they may, as indicated above, receive substance which escapes in a dissolved condition from the nucleus during every mitosis.

Whatever the ultimate origin or phylogenetic relationships of the centrosome may be, there is a remarkable parallelism between it and the nucleus, as the following statements will show:

1. Both begin their developmental cycle as small granules, the central corpuscle in the case of the centrosome, the chromosomes in the case of the nucleus.

2. Both grow enormously by the absorption of surrounding substances and become vesicular; in the cleavage of the egg the vesicular condition is followed by a reticular condition in both.

3. Both undergo radical changes in their staining qualities during this enlargement, passing from a condition in which they are uniformly chromatic to one in which they are almost entirely plasmatic in reaction; finally, they again become largely chromatic, so that the centrosomes in the resting stages of certain cells look like small nuclei filled with a chromatic reticulum (*cf.* figs. 69, 73, 74, 75).

4. When they have reached their greatest volume both are proportional in size to the size of the cell-body in which they are found; this probably indicates that the substances absorbed by both in their growth are derived from the cytoplasm.

5. In both, the daughter structures (centrosomes or nuclei) are but a fraction of the mother organ, the remainder of the latter passing sooner or later into the cytoplasm.

The centrosome thus repeats the history of the nucleus; at one period it takes up substances from the cytoplasm; when it has reached its greatest size the new centrosomes and central spindle arise within the mother centrosome from a part of

its substance, and the remainder of the latter passes back into the sphere and ultimately into the cytoplasm. It is evident from this description that, as in the case of the nucleus, so also in the centrosome there is a sort of diastole and systole, the phases of the one alternating with those of the other.

(*d.*) *Comparisons.*—I have found centrosomes, similar in all respects to these just described, in three species of *Crepidula*, and in *Urosalpinx*, *Illyonassa*, *Fulgur*, *Sycotypus*, and *Aeolis*. In structure and history the centrosomes in all these gasteropods are similar to those which have been observed in *Diaulula* (MacFarland, '97), *Unio* (Lillie, '98), *Thysanozoon* (Van der Stricht, '98), *Rhynchelmis* (Vejdovsky, '88, and Vejdovsky and Mrazek, '98), *Actinosphaerium* (R. Hertwig, '99), and *Haminea* (Smallwood, '01), while they bear many resemblances to those which have been observed in *Echinus* (Boveri, '01), *Ascaris* (van Beneden, '87, Boveri, '88, '01, Brauer, '93, Fürst, '98), *Sida* (Häcker, '93), *Salamandra* (Rawitz, '96, Niessing, '99), *Salmo* (His., '98), *Fulgur* (McMurrich, '96), *Limax* (Byrnes, '96 and '99, Linville, 1900).

Van der Stricht's interpretation of the relations of the centrosome to the attraction sphere in *Thysanozoon* seems to me most satisfactory, not only because he has for it the approval of Van Beneden and Boveri, but also because by it the various forms of centrosomes present in the animals named above and particularly the remarkable centrosomes of mollusca can be satisfactorily related to one another and to other forms. We need not here concern ourselves with the origin of the centrosomes of the first maturation; Van der Stricht believes that they arise from the nucleus, and this view is supported and extended by the recent observations of Schockaert (1900). Soon after its appearance the centrosome of *Thysanozoon* is differentiated into a central corpuscle and a medullary zone (*couche médullaire*). These two together constitute the centrosome of Boveri, the central corpuscle being his centriole. The medullary zone is homogeneous in structure, and no astral fibres penetrate it except at the time of origin of the new spindle figure; it is usually bounded peripherally by a dark line (in reality a sphere).[1] Around this is a clear area traversed at all stages by delicate astral fibres; this is the cortical zone (*couche corticale*), and it is possible that it is derived, in part, from the centrosome. Around this is the zone of astral rays, which sometimes may be subdivided into an inner dark and a peripheral clear zone. The centrosome and cortical zone continually enlarge, as the division advances, until they reach a great size. In the division of this centre, the central corpuscle first divides, usually into two, and the central spindle appears between the halves; the medullary zone then becomes bounded by granules, and this boundary gradually fades from view, though Van der Stricht believes that the entire attraction sphere persists and divides, thus giving rise to

[1] The clear zone which is so generally found around the central corpuscle is believed by many observers to be the result of destaining. Such a zone is produced by destaining yolk-spheres, nucleoli, etc. Fischer ('99) calls this "Spiegelfärbung." It will be observed that the manner in which these centrosomes stain, which have a dense periphery and clear central area, is the exact reverse of "Spiegelfärbung."

the daughter attraction spheres. The relations of the parts of the centrosome, the attraction sphere and the aster of *Thysanozoon* may be indicated as follows:—

Centrosome { Central Corpuscle, Medullary Zone, Cortical Zone } of Attraction Sphere.
Centrosome { Inner Zone, Peripheral Zone } of Aster.

The most important points in which my observations differ from those of Van der Stricht are the following:—

1. The peripheral boundary of the centrosome (medullary zone) is much denser and more deeply staining than in *Thysanozoon*.

2. During the rest stages in the cleavage the central corpuscle is represented by an enormous number of granules, only two of which form the new centrosomes.

3. Neither the medullary nor the cortical zones of the attraction sphere ever divide as a whole, but after the origin of the new amphiaster they are slowly dissolved in the cytoplasm.

4. The central corpuscle of one generation gives rise to the entire centrosome, *i. e.*, central corpuscle and medullary zone, of the next.[1] This is most plainly seen in the anaphase of the first maturation.

5. At no time do the astral rays traverse the medullary zone,[2] though new rays which are not part of the old system may arise within that zone around the new centers.

In *Diaulula*, according to MacFarland, the centrosome increases greatly in size from the prophase to the anaphase and a single granule appears within it. This granule soon divides into two which move apart and become the new centrosomes. The whole of the old centrosome is transformed into the new centrosomes and central spindle. The rays are inserted on the centrosome, not on the central granule. Even after the new spindle figure has reached a considerable size the rays continue to be centered on the figure as a whole.

My observations differ from MacFarland's only in one respect: The whole of the old centrosome is not transformed into the new spindle figure, but the latter arises *within* the old centrosome. This is plainly true of the maturation stages, corresponding to those which MacFarland has studied, and in the first three cleavages, but the case is not so clear in the later cleavages, as a glance at my figures 74–76 will show.

According to Lillie ('98) each centrosome in the prophase of the second matur-

[1] I am not quite certain whether this may not be involved in Van der Stricht's statement that the centrosome becomes differentiated into a central corpuscle and medullary zone. The fact, however, that he maintains a persistence of the attraction sphere leads me to suppose that he regards each medullary zone as derived from a preexisting one.

[2] In this important respect my observations agree entirely with those of Boveri and his pupils, MacFarland and Fürst, and differ from those of Van Beneden. Indeed, it may be doubted whether the term "medullary zone" should be applied to a structure which shows no radiations. Other considerations, however, render it extremely probable that the peripheral layer of the centrosome in gasteropods and the "medullary zone" of *Thysanozoon* are homologous.

ation division of *Unio* is composed of several large granules into which rays are inserted. In the metaphase these granules subdivide, and some of the fragments are distributed in the form of a sphere, the "inner sphere;" "one of the granules remains behind as the centrosome of the new inner sphere," but "a large part of the centrosome granules is changed into the red-staining substance of the sphere." In the anaphase the granules of the inner sphere, together with the peripheral accumulation of its ground substance, fuse into a continuous membrane. "The centrosomes are united to the membrane of the inner sphere by a few irregular threads which are not part of the system of radiations." Within the sphere the daughter centrosomes and central spindle arise.

Lillie emphasizes the fact that the inner sphere is not the centrosome, and he says that Boveri's "centrosome" is really the inner sphere, while his "centriole" is the real centrosome. He also holds that MacFarland's "centrosome" is really the inner sphere.

Lillie's conclusions seem at first sight to be very different from any of those mentioned above, and yet on consideration it will be seen to be rather a difference of terms than of facts. His "inner sphere" is undoubtedly homologous with Boveri's centrosome, his "centrosome" with the central corpuscle or "centriole" of Boveri. As to the genesis of these parts, I have never been able to observe the formation of the "inner sphere membrane" from granules derived from the central corpuscle as Lillie has done, nor have I observed the fragmentation of the central corpuscle and the transformation of these granules into the ground substance of the centrosome (substance of medullary zone), though in early phases the centrosome of *Crepidula* is irregular in outline, as if composed of closely connected granules. In all cases which I have observed the central corpuscle enlarges but does not fragment; its substance accumulates peripherally and forms a continuous membrane which *subsequently* is transformed into a layer of granules. If the central corpuscle of *Unio* were to remain a single structure, and were to continually expand, the result would not be unlike my observations. The one critical point in the comparison of Lillie's observations with those of other investigators, is to determine whether the whole of his "inner sphere" is derived from the central corpuscle; if it is, the differences are only matters of detail. It should be remembered that, according to Lillie, the inner sphere is itself a structure which sooner or later disintegrates and passes into the outer sphere or cytoplasm, and that it should disintegrate at different stages in different eggs is quite possible.[1]

In a later paper on the subject, Lillie ('99) says that the inner sphere enlarges very rapidly after the formation of the second polar body, and its substance gradually merges with the general cytoplasm. Its interior is occupied by the vesicular sphere substance at the nodes of which are deeply staining granules. In this respect there is considerable difference between *Unio* and *Crepidula*, for in the latter the inner

[1] I ought to add that I have had the pleasure of seeing Lillie's beautiful preparations, and they leave no ground for doubting the accuracy of his observations. Professor Lillie also personally assures me that he is quite convinced that the whole of the "inner sphere" is derived from the central corpuscle. (See also his recent work, 1900, p. 242.)

sphere remains much smaller and persists for a considerable period, while the outer sphere undergoes a metamorphosis similar to that which Lillie describes. The inner sphere is very faint and difficult to detect in *Crepidula*, and it may be that Lillie has overlooked it, or it may disintegrate sooner in *Unio* than in *Crepidula* (*cf.* my fig. 36 and Lillie's ('99) fig. 14).

Vejdovsky ('88) found in the egg of *Rhychelmis* immediately preceding the first cleavage, a hyaline sphere, the "Periplast," within which in the course of cell-division a new element, the "Tochterperiplast" appears; the latter divides into two spheres which represent the poles of the new spindle. During the first and second cleavages a new element, the "Enkelperiplast" arises within the "Tochterperiplast," while the "Mutterperiplast" degenerates or fuses with the cytoplasm. This was the first observation tending to show that the new centers arise endogenously within the old.

More recently Vejdovsky and Mrazek ('98) have confirmed and extended this account. They find at the poles of the first cleavage spindle, a large sphere at the center of which is a deeply staining granule, the "centrosome" (central corpuscle); this is surrounded by a hyaline sphere the "Tochterperiplast" (medullary zone), at the periphery of which the rays are attached; surrounding this is the "Mutterperiplast" (cortical zone). For the sake of uniformity we shall use the terms in parenthesis in place of Vejdovsky's terminology. As mitosis advances the medullary zone (Tochterperiplast) grows rapidly, becomes reticular or alveolar in structure and is bounded by a dense peripheral zone; the central corpuscle decreases in size and stains less densely, while radiations appear around it within the medullary zone. Around the central corpuscle a new medullary zone appears within the old one. The central spindle is formed, after which the new medullary zone divides. Vejdovsky and Mrazek consider that centrosome and periplast are persistent organs of the egg and that they represent a single whole. The entire periplast, however, does not persist, but the inner zones give rise to the outer ones which gradually disintegrate into the cytoplasm.

The resemblances between these observations and those which I have described in the preceding pages are very striking. The only important difference between my own observations and those of Vejdovsky and Mrazek is the following: A new medullary zone does not form around the central corpuscle before the latter divides, but only afterwards, *i. e.*, in the mollusks which I have studied the two daughter centrosomes are present before a new medullary zone is formed.

R. Hertwig has observed in *Actinosphærium* a large "spongy centrosome" within which a "reduced centrosome" (central corpuscle) appears and divides into two; the latter then enlarge to form new "spongy centrosomes," while the former "spongy centrosome" does not divide, but disappears in the cytoplasm. The resemblances in this case to those discussed above are too obvious to need comment.

In *Limax*, Byrnes ('99) has found centrosomes which in many respects resemble those of *Unio*, *Crepidula*, *Aeolis* and other mollusks. In the metaphase of the first maturation there is at each pole a group of central granules, within a clear area, which is surrounded by a broad, densely staining zone. In the anaphase the

central granules divide into two groups and a spindle appears between them and within the "centrosphere." From my own observations I am convinced that both the central clear area, with its contained granules and the denser zone which surrounds it, belong to the centrosome, which, when fully formed in the anaphase, consists of central corpuscles and medullary zone, the latter bounded by a narrow line or layer of granules. If this be correct the spindle in *Limax* arises *within* the medullary zone, as in many other cases.

Linville (1900) has observed a similar centrosome in *Limax* and other Pulmonates, around which is a cortical zone of radiating structure. He has not followed the metamorphosis of the centrosomes in detail, but his figures give evidence that the history of the centrosome in these Pulmonates is not different from what is known in other mollusks.

Finally, it seems quite possible to interpret most of the multitudinous forms of centers which have been described in the eggs of various animals in accordance with the Van Beneden-Boveri idea, as extended and defined by Van der Stricht, and particularly by Boveri (1901), provided that the remarkable changes in the structure of the centrosome from prophase to telophase be kept in mind. In mollusks the centrosomes are characterized (1) by the great breadth and density of the peripheral portion of the centrosome which, about the middle stage of mitosis, forms a dense ring or sphere surrounding a clear area, and which in all stages sharply separates the centrosome from the surrounding sphere, (2) by the fact that the centrosomes grow to an unusual size during mitosis, and (3) by the origin of the entire amphiaster of one generation within the centrosome of a preceding one. Possibly the second and third of these characteristics are the results of the first, since the sharp boundary of the centrosome at all stages make it unusually easy to recognize the great growth of the centrosome and also the place of origin of the new centrosomes and central spindles.

Boveri's ('01) masterful contribution on the nature of the centrosome reached me some time after my paper had been completed, and I have therefore been unable to make the extended use of it which I could have desired. In broad outlines my conclusions as to the centrosome are fundamentally like those of Boveri. The one most important point of difference between us is that Boveri considers the centriole as a differentiation of the centrosome, perhaps a continuous and persistent structure, around which a portion of the centroplasm always remains to form the new centrosome. On the other hand, I hold with R. Hertwig ('99) that the centriole gives rise by growth to a centrosome, within which a daughter centriole differentiates, *i. e.*, the centriole undergoes in its cycle of development a metamorphosis into centrosome and daughter centriole. In each generation the outer zone of the centrosome is thrown off, while the new centrosomes and central spindle come from the center of the old. There is thus a kind of endogenous formation of centrosomes, as Vejdovsky and Mrazek maintain.

Since receiving Boveri's paper I have carefully re-examined the critical stages in my preparations to see whether I could have overlooked an outer zone of centro-

plasm around the deeply staining body at the center of the aster. There is a faintly staining zone surrounding the central body in the prophases both of the maturation and cleavage divisions (see figs. 4–7, 26–30, 52–56, 70–72, 76), but this zone according to Boveri's definition, does not belong to the centrosome, since even at its first appearance (cf. fig. 70) it is traversed by radiations; furthermore, a study of consecutive stages shows that it develops step by step into the inner portion of the sphere. On the other hand, I believe that I have followed the central, deeply staining body through every stage of its growth and metamorphosis, having seen it not merely in the stages represented in the plates, but in thousands of others, many of which were carefully drawn. The result of this study convinces me that the small, deeply staining granule of the early prophase becomes the dense, spherical body of the metaphase and the large, hollow sphere of the anaphase, and that this body is the centrosome. The fact that in the mollusks generally the peripheral layer of the centrosome stains more densely than the central portion, makes it unusually easy in these animals to distinguish between the centrosome and the surrounding sphere. The result therefore of the re-examination of my preparations in the light of Boveri's work does not in any respect lessen my confidence in the accuracy of my observations and interpretations, at least as far as *Crepidula* is concerned.

The type of centrosome represented by *Crepidula*, *Unio*, *Haminea* and *Aeolis*, viz., one within which the new centers and central spindles arise from the centriole while a considerable part of the mother centrosome fades away into the sphere, agrees much more closely with the types of centrosomes found in *Ascaris*, *Thalassema* and *Echinus*, than does that of *Diaulula*. Boveri represents these four types in text figures (pp. 102–103), and it can be seen at a glance that in the first three types the daughter centers and spindles occupy but a small part of the old centrosome, whereas in the fourth type (*Diaulula*) they occupy the entire centrosome. I have found that the relative size of the central spindle and daughter centrosomes (Netrum of Boveri), as compared with the inclosing centrosome, differs considerably in different cleavages of the egg. Thus in the first, second and third cleavages the netrum is much smaller than the mother centrosome, whereas in the fourth, fifth and later cleavages the netrum almost entirely fills the mother centrosome. In view of these facts I venture to suggest that a re-examination of *Diaulula* with regard to this point might show that the outlines of the netrum are not coincident with those of the mother centrosome, but that the former lies *within* the latter as is the case in the other mollusks named above, as well as in other types of centrosomes described by Boveri.

(*e*) *The Centrosome as a Persistent Cell Organ.*—There is no more perplexing problem in connection with the cell than that of the significance of the centrosome. On the one hand there are the well established facts as to (1) its persistence from cell cycle to cell cycle (my own observations showing that in the cleavage of *Crepidula* it persists without interruption to a stage with more than sixty cells and probably throughout the entire development); (2) its independent growth and

division (shown not only by observation in many animals, but particularly by Boveri's ('97) experiments on echinoderm eggs); (3) its characteristic structure and metamorphoses, which in a large number of animals (perhaps in all) can be reduced to a common type.

These features are of such character and importance that they justly entitle the centrosome to the rank and title of a permanent cell organ (Van Beneden, Boveri). One who has followed the history of the cleavage centrosomes through several cell cycles, who has observed their unfailing persistence, the regular cycle of changes in form and staining reactions which they undergo, their complex structure, their form of division, their parallelism in these and in other respects to the nuclei (see p. 55), can no more doubt that these centrosomes are persistent cell-organs than that nuclei or plastids are.

On the other hand there are the well known facts (1) that, according to the best testimony, there are no centrosomes whatever in the higher plants (Strasburger, Osterhout, Mottier, *et al.*); (2) that the persistence of centrosomes has been denied in the tissue cells of some animals, and even in certain stages of the egg, particularly during fertilization (Foot, '97, Lillie, '98, Child, '99); (3) that various stages intermediate between centrosomes and microsomes or other cytoplasmic constituents have been described (Bürger, Reinke, Watase, Mead, Eismond, Erlanger) which indicate that the centrosome is only a temporary differentiation of the cytoplasm; (4) that artificial asters and centrosomes may be formed in egg cells by the action of various solutions, and that these may function as normal asters and centrosomes (R. Hertwig, Morgan, Loeb, Wilson).

The contradiction between these two classes of evidence is so complete, and the phenomena in both classes are apparently so well attested, that one would be inclined to seek refuge in the conclusion that in some cases the centrosomes are persistent cell organs and in others temporary structures, were it not for the fact that this contradicton may concern one and the same object (*e. g.*, the eggs of Echinoderms and of *Chætopterus*).

There is certainly no ground to doubt that in the cleavage of the eggs of many animals the centrosomes are, under normal conditions, absolutely continuous from cell generation to cell generation. Nor is there any possibility of doubting that in certain animals the centrosomes show independent growth and division, and that they pass through certain characteristic metamorphoses in this cycle. The only possible interpretation of these undoubted facts is that, in some cases at least, the centrosome is a cell organ of morphological as well as of physiological significance.

Is the contrary evidence irreconcilable with these well established facts, and must we, therefore, conclude that the persistence of centrosomes, their growth, metamorphoses and division have no general morphological significance? I think not.

(1) If it be granted that the centrosomes are not present at any stage in the cell cycle in the higher plants, this does not necessarily contradict the centrosome theory of Van Beneden and Boveri, since the fact that they are present in the lower plants indicates that their absence in the higher plants must be the result of degene-

rative changes. If, however, centrosomes may degenerate in whole classes of the plant kingdom, the centrosome is surely neither so ubiquitous nor so necessary a cell organ as the nucleus.

(2) So far as animals are concerned the centrosome has been found in almost all kinds of metazoan cells, and at nearly every stages of the cell cycle. The history of biology shows that the failure to find structures, even by many observers, is no proof that they do not exist, and this is particularly the case with structures so difficult to observe and undergoing so great metamorphoses as the centrosomes. As to the alleged disappearance of the centrosome in the fertilization of the egg (Foot, Lillie, Child,), it must be said that this like negative evidence in general is not wholly conclusive. Certainly, so far as my own work goes, I cannot affirm that both egg and sperm centrosomes entirely degenerate, although they do disappear, nor can I affirm that the cleavage centrosomes are new formations, although I am unable to trace any connection between them and the centrosomes of the egg and sperm. Even if these centrosomes disintegrate, it may be that the new centrosomes arise from some of their fragments; in fact, such would seem to be the case in *Crepidula* (see p. 27). The history of the centrosomes in the fertilization is at best a complicated one, and is by no means as clear as in the cleavage of the egg or in the division of tissue cells, and until we have more exact knowledge of the origin of the centrosomes in the fertilization, this doubtful evidence against the continuity of the centrosomes should not be permitted to outweigh the positive evidence in favor of their continuity afforded by ordinary mitoses.

(3) The view that the centrosome is only the meeting point of astral rays or that it represents merely a condensation of the cytoplasm, or that it is an enlarged microsome, entirely neglects to take account of the complex structure and metamorphoses of the centrosome, as well as of its division and persistence. These are by all odds the most characteristic features of a centrosome, and until it has been shown that the cytoplasmic structures mentioned above are capable of reproducing these characteristic features, it may well be doubted whether they are really centrosomes. The mere formation of cytoplasmic radiations is in itself no positive indication of the presence of a centrosome, since such radiations are found in the higher plants where centrosomes are wholly lacking (Osterhout, Mottier), in non-living substances such as carbolic acid and chloroform, gelatin and albumen (Roux, Bütschli, Fischer), where there is certainly no centrosome with the characteristics described above; around mid-bodies (see figs. 60, 61), and in many of the multiple and accessory asters found in cells under normal and artificial conditions, which show no body at the center of the rays (Mead, Lillie, Morgan, *et al.*).

(4) The fourth class of facts which speak against the theory of the persistency and morphological importance of the centrosome forms by all odds the most serious objection to that theory which has yet been raised; I refer to the experimental production of centrosomes both in fertilized and in unfertilized egg cells by the action of various solutions (R. Hertwig, Mead, Morgan, Loeb, Wilson). It may well be doubted whether all of these structures are centrosomes, but that some of them

are such is beyond dispute. Certainly, structures which function as centrosomes through a long period leading up to the production of larvæ (Loeb, Wilson), are enough like centrosomes to pass under that name. And even in cases where larvæ are not produced (experiments of Hertwig, Mead and Morgan), there can be no reasonable doubt that centrosomes are found in the larger asters, even if the smaller ones do not contain them.

In the case of Morgan's experiments on fertilized eggs it might be maintained that the numerous asters and centrosomes observed are derived by division or fragmentation from those already present in the cell-body, where it not for the fact that similar asters and centrosomes have been observed in the case of unfertilized eggs (Hertwig, Morgan, Wilson) where no centrosomes are present in the cytoplasm. The phenomena in these two cases are so similar that one cannot believe that they are due to wholly different causes; we may, therefore, safely class them together.

Hertwig maintains that in his experiments the centrosomes were formed from the achromatic constituents of the nucleus. He says: "Ich deute somit die Centrosomen as selbständige gewordene geformte achromatische Kernsubstanz, eine Deutung für die ich wiederholt eingetreten bin." In part Morgan agrees with this position, though he also holds that centrosomes may arise at a distance from the nucleus and therefore from the cytoplasm. "I agree," he says, "with Hertwig that the centrosomes may develop out of the achromatic substance of the nucleus, but I see no ground to extend this statement to include all centrosomes. . . . There is good evidence to show, I think, that similar bodies may arise in the cytoplasm also, as shown by Reinke, Mead, Watase and myself." It is a notable fact, however, that in all these cases cited by Morgan the nuclear membrane has disappeared or is much shrunken and collapsed, showing that nuclear substance has escaped from it. This is true at least of the figures of Reinke, Mead and Morgan; Watase gives no figures of the egg of *Macrobdella* which he describes as containing "a series of thirteen asters, ranging from the miniature aster, with the microsome in its center, to the normal aster with a veritable centrosome." In the figures of Reinke, Mead and Morgan one is much struck by the fact that at the time when the asters appear in the cell the nuclear membrane is either entirely lacking or is much shrunken, showing that achromatic material has escaped into the cell. Of his own experiments Morgan says (p. 464): "The first effect (of the salt solution on the egg) is to cause a shrinkage of the nucleus; then after the return of the eggs to sea water the division of the nucleus and subsequently that of the egg takes place; . . . central bodies are present in the artificial astrosphæres in almost all the stages." Again (p. 517) he says: "At the time when the nuclear wall disappears the astrosphæres throughout the egg, whether in contact with the chromosomes or not, become conspicuous and then fade away again as the chromosomes pass into the resting nuclei. There is some connection between the setting free of the chromosomes and the development of the astrosphæres;" or rather, as it seems to me, between the escape of some nuclear constituent and the development of the astrosphaeres. The fact that achromatic nuclear substance may be distributed widely through the cell in normal mitoses was pointed out in the section on aster

formation, and I see no evidence in the cases brought forward by Morgan to indicate that the centrosomes or asters in all these cases may not be derived from escaped nuclear material.[1]

There is certainly a close relationship between the nuclei and the centrosomes. The achromatic substance of the nucleus contributes to the growth of the centrosome in every normal cell cycle (see p. 54), and it is probable that the daughter nuclei in their growth resorb from the spheres a portion of this same achromatic substance. The peripheral spindle fibres are formed out of this substance (*viz.*, linin and oxychromatin), and bear a striking resemblance to the central spindle fibres at an early stage (*cf.* figs. 55 and 75). In the first maturation of the egg the centrosomes or asters do not appear until substances have escaped from the nucleus, as is shown by the breaking or indentation of the nuclear membrane (*cf.* Coe '99, Carnoy and Lebrun '99, Gardiner '98, Griffin 1900, Mead '98, Van der Stricht '98, Schockaert 1900), and, finally, the granular or reticular centrosome undergoes the same changes in reaction to stains as does the oxychromatin and linin, being at one time uniformly chromatic, and later uniformly plasmatic in reaction. In all these respects the centrosome behaves like an isolated portion of the oxychromatin and linin.

A large number of investigators have observed the formation of centrosomes and spheres from some of the nuclear constituents, particularly among the Protozoa, (Brauer '93 in *Ascaris*, Rückert '94 in *Cyclops*, Ishikawa '94 and Calkins '98 in *Noctiluca*, Balbiani '95 in *Spirochona*, Schaudinn '96 in *Acanthocystis*, Hertwig '99 in *Actinosphærium, et al.*).

[1] Wilson's ('01) recent work on *Toxopneustes* shows that asters and centrosomes may arise in eggs treated with $MgCl_2$, not only far from the nucleus, but even in enucleated fragments. Wilson says (p. 542) "There is absolutely no evidence for, and the clearest evidence against, the view that the original cytasters form at or near the nucleus, to migrate thence toward the periphery, or that they arise by the multiplication of a single primary center." He holds, therefore, that centrosomes and asters may arise *de novo* in the cytoplasm. Such a view, if generally true, would be fatal to the one which is set forth in this paper, and it deserves more extended treatment than can be accorded to it in a foot-note. In brief, the critical questions as to Wilson's experiments are these: (1) Are the bodies in question real centrosomes; (2) do they arise *de novo* in the cytoplasm? I am not disposed to question the fact that these bodies are really centrosomes, but I am inclined to doubt the statement that they arise *de novo*, if by that it is meant that they arise without genetic relation to other centrosomes or to the nuclei. The fact that these "artificial" centrosomes may appear far from a nucleus, or even in enucleated fragments, does not necessarily imply that they are wholly independent of them. The achromatic substance of the nucleus may be widely distributed throughout the cell during mitosis, and I have observed in the eggs of *Crepidula*, which have been placed in 2%-3% Na Cl for several hours, that the achromatic portion of the nucleus may exist as one or more vesicles, with definite walls, quite distinct from the chromatic portion. In some cases these achromatic vesicles are in contact with the chromatic one; in others they are widely scattered throughout the entire cell. *Furthermore, many of these vesicles apparently give rise to centrosomes.* If the achromatin of the nucleus is genetically related to the centrosome as I have maintained in this paper, and if achromatin, diffused throughout the cell, may, under certain stimuli, be aggregated into vesicles, which then give rise to centrosomes, Wilson's observations need not necessarily mean that centrosomes arise *de novo*.

In all cases in which "artificial" asters and centrosomes have been produced, a large amount of nuclear substance has been present in the cytoplasm. No one, so far as I can recall, has observed asters in egg cells while the germinal vesicle is still intact; with the escape of achromatin from the germinal vesicle, however, numerous asters and possibly centrosomes may appear in the egg. I have tried by various means to produce asters in egg cells before maturation, but always without success as long as the germinal vesicle remains intact. I believe that it may be laid down as a general principle that *escaped nuclear material is essential to the formation of an aster, and that an aggregation of such material is necessary to the formation of a centrosome.*

Others have observed that centrosomes disappear within the growing daughter nuclei in certain cases. For example, Mead says that the centrosome left in the egg at the close of the second maturation of *Chætopterus* is last seen "in the midst of the fusing (chromosomal) vesicles, its position being indicated by the point of convergence of the rays of its waning aster." Exactly the same thing is true of *Cerebratulus* (Coe), *Thalassema* (Griffin), *Asterias* (Wilson and Mathews) and probably of other animals. In all these cases the centrosome is probably taken into the egg nucleus.

All of these facts seem to me to indicate that the centrosome is intimately related to the "formed achromatic" substance of the nucleus and that, in some manner, artificially produced centrosomes are formed out of this material as R. Hertwig maintains.

Loeb has found, by a series of remarkable experiments, that artificial parthenogenesis may be caused in the eggs of echinoderms and of *Chætopterus* by the action of a variety of substances upon the eggs, and he concludes that in general this parthenogenesis is the result of diosmotic action of these substances and the withdrawal of water from the egg, though other factors also enter into the problem in the case of *Chætopterus*. In the light of the many observations and experiments which go to show that asters and centrosomes are produced from escaped nuclear material, the thought suggests itself that artificial parthenogenesis may be caused by any method which will bring certain nuclear constituents into the cell body and yet not seriously injure either nucleus or cytoplasm.

One of the most interesting chapters of Boveri's recent work on the centrosome is that in which he undertakes to account for the production of centrosomes by artificial means. Boveri recognizes, as have many others, the intimate relation between the achromatic material of the nucleus and the centrosome. In cases where real centrosomes can be produced from unfertilized eggs he holds that they are formed by a kind of regeneration (*reparation*, Driesch '97) from the achromatic substance of the nucleus. Not all nuclei, however, have this power, and, accordingly, Boveri distinguishes between (1) *nuclei* which are purely nuclear in character, and (2) *centro-nuclei* which contain a cytocenter. An example of the former is found in *Ascaris*, and of the latter in many Protozoa, and in some Metazoa, particularly in the echinoderms and in the ovocytes of many animals—perhaps of all.

The question at once arises: What reason is there for supposing that among Metazoa nuclei are divided into these two classes? Boveri himself has asked this question, and he concludes that in *Ascaris* at least the nucleus cannot be a centronucleus, since in certain pathological eggs, in which the spermatozoon remained at the periphery or did not enter at all, the egg went through the maturation divisions and the egg nucleus came to the period of the solution of the nuclear membrane without a trace of fibre differentiation, of centrosomes, or of spindles or spheres. He therefore concludes that the nucleus of *Ascaris* is a pure nucleus which has lost the capacity of forming centrosomes.

The evidence upon which such an important generalization is based seems to

me to be insufficient. No small amount of evidence is required to prove that nuclei, which are so similar in all other respects, are so different in this one. I agree with Boveri that research only can determine where (and I should add whether) this is true.

Returning now to Boveri's idea that in the artificial production of centrosomes they are formed by a kind of regeneration:—Morgan's experiments make it extremely probable that numerous centrosomes may be formed independently of each other in the cytoplasm. If these are formed of achromatic nuclear material it is easy to understand that they appear wherever a sufficient accumulation of this material is found in the cell body. But achromatic material separated from the nucleus is not necessarily a centrosome with all of the morphological and physiological features which that body exhibits, as is shown by the fact that such material is distributed through the cell at every mitosis. Either there must be an escape of some centrosomal substance or structure, or the condition of the cell must be such as to bring about centrosome formation from ordinary achromatic material; the latter is I believe Hertwig's view (see quotation on p. 64); the former is held by Boveri who considers that the centrosome may be regenerated (repaired) from the achromatic substance of centro-nuclei only.

This very suggestive hypothesis of Boveri's makes it possible to harmonize the well established fact of the persistence of the centrosome as a cell organ with that other apparently contradictory fact that centrosomes may be produced experimentally in the cell; and this it does by practically adding another phase to the series of changes through which the centrosome may pass, *viz.*: the phase of the centro-nucleus.

But the question of the persistence and morphological significance of the centrosome does not hang on the fate of the uncertain hypothesis that nuclei belong to two classes, pure nuclei and centro-nuclei. The one point of importance is that centrosomes are not coagulation products, nor the mere expression of cell stresses, nor sporadic or spontaneous structures, which may appear and disappear here, there or anywhere, depending upon the physiological condition of the cell; but that all kinds of centrosomes, whether normal or artificial, are formed of a specific kind of protoplasm which is genetically related to the achromatic substance of the nucleus, from which, under certain conditions, they may be formed anew, that they have a characteristic structure and metamorphosis, that they possess the power of independent growth and division, and that they are therefore cell organs, which are at least relatively, even if not absolutely, persistent structures of high morphological significance.

(*f*) *Homology of the Centrosome.*—We may now again consider the parallelism between the nucleus and the centrosome pointed out in a previous section (p. 55). This parallelism is seen not only in the alternate growth and diminution of both, but also in corresponding changes in staining reactions and in similarities in their modes of self propagation, both nucleus and centrosome being continued from generation to generation by means of small granules (chromosomes, central corpuscles) which have the power of independent growth and division.

What is the significance of this parallelism between the nucleus and the centrosome? Does it indicate that these two structures are genetically related or may it be due to simpler physical or physiological factors? The parallelism in the *growth and diminution* of these structures indicates that both nucleus and centrosomes are diffusion centers which alternately enlarge by the absorption of substances from the cell body and diminish by the return of substances to the cell body. It is possible that their parallel changes in *staining reactions* are the results of the absorption of similar materials from the cell body, or it may be due to the fact that the centrosomes imbibe a certain amount of material which escapes from the nucleus. The fact that they are both *self propagating* is a property which they share in common with other cell constituents (*e.g.* plastids) with which they are certainly not homologous.

On the other hand the remarkable manner of this self propagation is shared, I believe, by no other cell constituents. Moreover, the singular resemblance between the reticular central spindle (netrum), the achromatic reticulum within the nucleus and the intranuclear spindle of many of the Protozoa is most striking and finds no satisfactory explanation along the lines just indicated.

Many persons who have worked upon nuclear division in the Protozoa (*e.g.* Bütschli ('91), R. Hertwig ('92, '99), Schaudinn ('95, '96), Lauterborn ('96), Calkins ('98) have pointed out the resemblances between micro-nucleus or the intra-nuclear mitotic spindle and the centrosome of the Metazoa, and this homology has been maintained with great force by Heidenhain ('94) and Boveri ('01).

R. Hertwig in particular has repeatedly advocated this homology. In *Actinosphaerium* he has observed that the centrosomes are actually budded out of the nuclei, and he concludes that the centrosomes are to be considered an escaped achromatic substance of nuclear origin, "nuclei without chromatin." He has also pointed out the steps by which, he thinks, the evolution of the centrosome has taken place, as well as the phylogenetic relationships of the various types of centrosomes to each other and to the nuclear structures of the Protozoa.

Heidenhain also has forcibly presented the resemblance between the central spindle of the Metazoa and the micro-nucleus of the Infusoria. He concludes that the two are homologous, that the centrosomes of the Metazoa are only polar differentiations of the intra-nuclear spindle of the Infusoria, while the macro-nucleus of the latter corresponds to the nucleus of the Metazoa; the chromatic substance of the micro-nucleus has disappeared in the Metazoa, being transformed into the archoplasm zone.

These ideas of Heidenhain called forth the severe criticism of Boveri ('95) who held that since the Infusoria cannot possibly represent the ancestors of the Metazoa, the nuclear structures and functions which occur in these cannot properly be considered the prototypes of those in the Metazoa. Further, he held that the macronucleus was probably a transformed micro-nucleus, and that actual, independent centrosomes were present in some Protozoa.

It is interesting to find that in his recent work on the centrosome Boveri ('01),

while still maintaining some of the propositions named above, acknowledges that his opposition to the view that the intra-nuclear spindle of the Protozoa is the homologue of the metazoan centrosome was not justified. The fact that so careful and far seeing an investigator as Boveri should find cause to reverse his position on this question lends additional weight to this idea.

Boveri compares in detail the micro-nucleus of a ciliate infusorian in the spindle stage, the ovocytic spindle of *Ascaris*, the nuclear spindle of *Opalina*, which does not fill the whole of the nuclear cavity, the ovocytic spindle of *Diaulula*, which lies entirely outside of the nuclear cavity and in which centrosomes have differentiated at the poles of the spindle, and finally a type such as *Ascaris* (cleavage stages) in which the central spindle connecting the centrosomes has almost entirely disappeared. In this series is shown the supposed steps by which the centrosome is differentiated at the poles of the spindle-shaped figure (netrum) and by which the latter comes to lie outside of the nuclear cavity and separate from the chromosomes.

How entirely my observations on *Crepidula* and other gasteropods accord with the general homology suggested by these different investigators can be seen at a glance at my figures. Compare for example the cleavage centrosomes of *Crepidula* (text fig. IV and Plate IV, figs. 69–76) with the micro-nucleus of *Paramœcium* (Hertwig):[1]

(1) In the resting stage both are reticular spheres; (2) As the division begins they become spindle shaped and the reticulum is drawn out into spindle fibres; (3) At the poles of this spindle pole bodies (plates) appear, the spindle and pole bodies forming a unit structure (Heidenhain); (4) In this process the centrosomes of *Crepidula* repeat some of the very stages which the authors named above assume to have occurred phylogenetically, *i. e.*, the reticular spindle when first formed shows no sharply differentiated body at its poles; later a centrosome appears at each pole, whether as a new differentiation or from a granule before indistinguishable from the others I cannot decide.

In one respect, however, there is an important difference between the centrosomes of these gasteropods and the micro-nuclei of *Paramœcium* or other Ciliata, *viz.*: in the former the new spindle figure (netrum) is formed within the old centrosome, the outer zone of which disintegrates. This resembles the observations of Hertwig on *Actinosphaerium*, but is unlike the division of the micro-nucleus of the Infusoria where the membrane persists throughout the division.

On the other hand the disintegration of the centrosomal membrane in gasteropods precisely resembles the behavior of the nuclear membrane among Metazoa; this membrane which is composed of substances similar to, if not identical with the formed achromatic substance (oxychromatin and linin) dissolves and disappears during mitosis, just as the centrosomal membrane does. Both nuclear and centrosomal membranes, like their contents, undergo similar changes in staining reactions

[1] There is also a striking resemblance between the form of division of the central corpuscle in the first maturation of *Crepidula* and the division of the "*Nebenkörper*" in the swarm spores of *Paramœba* (Schaudinn '96).

during the cycle of division, *i. e.*, both are chromatic at one stage and plasmatic at another. I have already remarked upon the fundamental similarities between the nuclear reticulum and the centrosomal reticulum, and also upon the parallelism in the cycle of changes which both structures undergo. While therefore I recognize, together with the authors named above, the resemblance of the metazoan centrosome to the intra-nuclear spindle of the Protozoa, *i. e.*, the micro-nucleus minus the chromatin, I maintain with R. Hertwig that one need not go so far as the Protozoa to find structures homologous with the centrosome since such may be found in the formed achromatic substance of metazoan nuclei, *i. e.*, in "nuclei without chromatin" (basichromatin).

3. Spheres.—The large, densely staining sphere which persists through the whole of the resting stage in all of the gasteropods which I have studied, represents in the main the cortical zone of the attraction sphere, though remnants of the outer zone of the centrosome may be found in it after the origin of the new amphiaster. In each cell-generation the new centrosome and, perhaps, also the new cortical zone arises within the old centrosome (*cf.* figs. 25 and 70), so that the *anlagen* of both centrosomes and spheres (at least in some mitoses) come from the previous centrosome. Achromatic substance from the nucleus plus hyaloplasm from the cell body fills the cortical zone and swells it into the enormous sphere of later stages of division. After the new amphiaster appears it moves out of the old sphere, and the latter may persist for a long time as a degenerating structure.

In the eggs of all animals spheres are usually present during mitosis,[1] but they usually disappear at the close of division, and in no other case can I find any account of bodies with so compact a structure persisting throughout the whole rest, or even through the following mitosis.[2]

In spermatogenesis, however, bodies undoubtedly similar to these spheres have been described by several authors, particularly by Moore, '93, Meves, '94, '96, '98, Rawitz, '96. Meves finds in the resting spermatogonia of *Salamandra* at the end of summer that the sphere becomes a heap of granules; in the spring the sphere is reconstituted out of these granules and in the summer a "consolidated sphere" bounded by a sharp line or even a membrane is found. This sphere probably contains a centrosome at all times, though it is not always visible. The daughter centrosomes and central spindle arise within this sphere. The sphere is composed of a cortical and a medullary zone, and the former, at least, breaks up and is scattered through the cell. Masses of these granules collect close under the cell wall, and at the equator of the cell, and when the cell body divides they lie along the newly formed cell membrane. These granules are frequently present for some time in the daughter cells, but usually disappear before the next division. In all stages of this

[1] Carnoy and Lebrun ('97) totally deny the existence of spheres of any kind. They say: "Boveri's archoplasm is a part of the cell-substance; van Beneden's attraction sphere, Guignard's directive sphere, Vejdovsky's periplast, the earlier kinoplasm of Strasburger do not exist as such. These myths belong in the legends." . . Such sweeping statements are chiefly valuable as illustrating the worthlessness of dogmatic biology.

[2] Bolles Lee ('95) finds in the testis cells of *Helix* that the spindle remnants are preserved from generation to generation, the old spindle remnants fusing with the new ones.

metamorphosis the sphere substance remains distinct from the remaining cell substance and in this respect resembles the archoplasm of Boveri, though spindles are not formed out of it. It has been known variously as "sphere," "*Nebenkern*," "*Centrodeutoplasm*" (Erlanger); Meves proposes to call it *Idiozome*. It is formed from eliminated chromatin; as to its origin he says:—(Meves '94, p. 158) "Einen anderen Theil des eliminirten Chromatins, der nicht zur Bilding eines Nebenkerns verwandt wird, findet man in den Spermatogonien des Salamanders nach meiner Beschreibung in dem die Sphäre representirenden Körnerkranz; in einem bestimmten Stadium des Processes sind kleinste Chromatinkügelchen von einem Hof von Spärensubstanz umgeben." Again (p. 159):—"Später scheint das Chromatin seine Reaction zu ändern und in dem Aufbau der Sphäre mit einzugehen."

The idiozome is found particularly, perhaps exclusively, in male sexual cells, and Meves agrees with Kostanecki and Siedlecki, Erlanger, Lenhossek and Montgomery, that it cannot be homologized with the sphere of egg cells, though, perhaps, it is homologous with the yolk nuclei of ovarian eggs. Unlike the attraction sphere of van Beneden it is (1) not radiating in structure, (2) only present in resting stages, not in mitosis, (3) sharply limited from surrounding cytoplasm. In spite of these differences I make bold to say that the spheres which I have observed in gasteropod eggs are certainly homologous with Meves' idiozome. To recall only a few of the resemblances:—(1) Both arise (in part) from eliminated chromatin which changes its staining reaction. (2) Both have the same relations to the centrosomes and central spindle. (3) Both persist and preserve their individuality through the entire resting period and even into the following mitosis. (4) Both show a cortical and a medullary zone. (5) Both are sharply delimited from the cytoplasm. (6) Neither has radiating structure during the rest stage. (7) Both ultimately disintegrate and are scattered as coarse granules immediately under the cell membrane.

The sphere in these gasteropods is derived from an undoubted attraction sphere, with radial structure and without sharp demarkation from the cytoplasm. I conclude, therefore, that the term *idiozome* should be extended so as to include the spheres present in all resting cells, or that it should be abandoned altogether. Since it represents, in gasteropod eggs, merely the resting stage of the sphere, it seems to me no more entitled to a specific name than the resting nucleus.[1]

4. Archoplasm.—Of late there has been much discussion of the Archoplasm hypothesis of Boveri ('88) and the general conclusion seems to be that this hypothesis is untenable (*cf.* Heidenhain, Kostanecki, Erlanger, Wilson, R. Hertwig, Carnoy and Lebrun, *et al.*) This conclusion is based on the proposition that there is no peculiar substance such as Boveri's hypothesis supposes. Boveri ('95) himself admits that he has been unable to find archoplasm except in the eggs of *Ascaris* and in *Noctiluca*.

The principal features of Boveri's archoplasm hypothesis are the following (*cf.* Wilson, '95, p. 444): — (1) A specific substance, distinct from other cell constituents,

[1] R. Hertwig ('99) expresses the view that idiozome, spheres, centrodeutoplasm, Nebenkern, etc., are only giant centrosomes or centrospheres.

which may be scattered through the cell, or aggregated into a sphere around the centrosome. (2) In karyokinesis it divides, following the division of the centrosome, and it forms the achromatic spindle and the polar astral systems, its granules being transformed into fibres. (3) After karyokinesis its fibres are again resolved into granules which are withdrawn into the spheres. (4) It is a persistent cell element.

The many points of resemblance between this substance and the "sphere substance" of *Crepidula* must be at once apparent; the most important points of difference are that in *Crepidula* and other gasteropods this substance does not divide with the division of the centrosome and is not a self perpetuating cell element. It is a specific substance, temporarily distinct from other cell elements; it arises anew in each cell generation; it forms a part of the spindle and asters; at the close of karyokinesis it is withdrawn into a sphere surrounding the centrosome, and when the intial spindle moves out of the sphere, the latter slowly disintegrates and disappears in the general cytoplasm.

Boveri held that the archoplasm sphere was largest in the prophase and then diminished in size as rays were formed out of it. Zeigler, Kostanecki and Seidlecki find that the reverse is the case, *viz.*, that the granular mass increases as the rays increase and decreases with them. In all the gasteropods which I have studied with reference to this point, this substance is smallest in quantity at the beginning of karyokinesis and continually increases as division advances.

If regard be had to the exact definition of archoplasm which Boveri gave, then this sphere substance cannot properly be called archoplasm. However it sufficiently resembles the archoplasm in location and general characteristics to warrant the belief that it corresponds to the substance observed by Boveri, if not to his definition of that substance.

A specific substance, at least temporarily distinct from the general protoplasm of the cell has been found in astral and karyokinetic figures by almost all recent writers; *cf. Kinoplasm* (Strasburger), *Ergastoplasm* (Prenant), *Cyanoplasm* (Morgan), *Archoplasm* (Wilson, Griffin, Foot and many others). The whole appearance of a karyokinetic figure, its definite form, separation from the surrounding cytoplasm, staining reactions, all show that we are here dealing with a substance which is specifically different from the general cytoplasm. The physiological relations of the amphiaster to the cell body, no less than its morphological characteristics lead to the same conclusion. On the other hand the evidence both from observation and experiment now renders it extremely probable that this specific substance is a temporary differentiation of the cell- and nuclear-plasm which may again be transformed into the general protoplasm; in short it is not self propagating and absolutely continuous.

Those who have studied the eggs of *Ascaris* can scarcely doubt that the substance which Boveri described in that animal under the name of *Archoplasm* is homologous with the specific substance found in the astral and karyokinetic figures of many other organisms, and if this be the case it seems to me that the name given

by Boveri, or, rather, the modified form of it, viz., *Archiplasm*, which has been widely used and accepted, ought still to be applied to this substance, even though Boveri's views as to its characteristics may not be fully accepted in all cases. Fortunately it is not generally considered necessary to change the name of a thing every time we change our views as to its qualities. Neither the cell itself nor any morphological element of it means to all investigators the same thing which it meant to its discoverer, and yet we generally find it advisable to retain the old name, changing our conception of the thing named as our knowledge advances.

II.

CYTOKINESIS.

The term *Cytokinesis* (Whitman, '87, Ryder, '94, Rhumbler, '96) is here used to designate those movements within the cell body during its cycle of division which correspond to the nuclear activities during the division of the nucleus. Viewed in this light, cell division consists of karyokinesis and cytokinesis, the two being so intimately related that one cannot be treated wholly apart from the other.

These protoplasmic movements are of particular interest in that they throw light upon the constitution of the cell and the mechanics of cell division as well as upon the more complex problems of differentiation. Thus the movements in the spindle, aster and cell body during division indicate that the protoplasm is of a fluid or semi-fluid constitution, permitting freedom of movement among its parts; that these movements are in the nature of diffusion streams, as Bütschli has repeatedly maintained; and, further, that these movements are the immediate cause of many important differentiations.

Before taking up these movements in detail we shall briefly consider:

I. The Structure of the Cytoplasm.

The cytoplasm of the egg of *Crepidula* presents the appearance of being composed of alveoles, and this conception of its structure is most in consonance with the many movements within the cell which will be described later. The egg contains a large quantity of yolk in the form of spherules which vary enormously in size. The smaller yolk spherules seem to lie within the alveoles, though the larger ones of course do not. In addition to the yolk spheres which always stain intensely with iron haematoxylin, there are other small spherules which are never seen except in material fixed for a considerable period in Hermann's fluid. These spherules are about the size of the smallest yolk spheres and like them appear to lie within the alveoles, but unlike them do not stain with haematoxylin and are of a pale gray color. They may represent partially dissolved or digested yolk spheres.

With the highest powers of the microscope which I have been able to use, *viz.*, Zeiss Apochromatic Obj. 1.5 mm. Occ. 12, the karyokinetic spindles and astral rays show no indication of alveolar structure, nor indeed does the nuclear sap which forms the interfilar substance of the spindle and spheres. In the anaphase and telophase, the spheres as well as the centrosomes are alveolar or recticular. Within the cytoplasm the alveoles are smaller and their walls thicker the nearer they lie to the centrosomes (figs. 54, 55) as Bürger ('92), Eismond ('95), Rhumbler ('96) and Erlanger ('96), have shown to be the case in other forms.

In certain stages (*e. g.*, fig. 54) the walls of the alveoles are very thick and stain so deeply that I conclude that they are composed of achromatic nuclear material which has diffused from the spheres in addition to the substance of the alveolar layer (hyaloplasm) which is diffusing toward the spheres from the cell body. These radiations run between the alveoli and not through them, and in certain stages, particularly early prophases and late telophases, show a zig-zag course between alveoli or even branchings and anastomoses around them, figs. 54, 61, *et seq.* (*cf.* Wilson '99).

How to harmonize the well known fact that protoplasm behaves as a thick fluid, with those other well established facts as to its differentiations and the localization of differentiated structures within it, is a problem to which much attention has been paid. Long ago Brücke ('61) pointed out the fact that a definite organization could not exist in a fluid and that a fluid plasma would be incapable of performing the complex functions of the cell. Since that time the further study of the cell has but emphasized the great complexity of its structure and functions, and this has led many authors to regard the cell as a relatively stable system of parts, the interstices between which are filled with a fluid-like substance. On the other hand the continued study of living protoplasm has more fully demonstrated its fluid character; the freedom with which parts may move about within cells, particularly in certain Protozoa and in metazoan egg cells, is entirely inconsistent with the idea that the cell is traversed by a fixed system of fibres which bind all its parts into a stable system. Bütschli's theory of the structure of protoplasm is the only one which undertakes to harmonize these apparently contradictory phenomena, for while emphasizing the fluid character of protoplasm it still assigns to it a definite structure and provides for the local differentiations and specific organization of the cell (*cf.* Bütschli '92, Rhumbler '98). I accept without reserve the Bütschli theory so far as it concerns the general cytoplasm of the eggs which I have studied, but I have seen no sufficient evidence that it extends to all the parts of the cell.

II. Movements of Cell Contents.[1]

In the maturation, fertilization and cleavage of the gasteropod eggs which I have studied I have observed successive stages of a complex and orderly movement of the entire cell substance by which the positions of the cytoplasm, yolk, nuclei, centrosomes, spheres and mid-bodies (*Zwischenkörpen*) are changed in a definite and orderly way. Unfortunately, I have been unable to actually observe these movements in the living egg, since the eggs studied contain a large amount of yolk and are therefore opaque, and since the movements described are very slow. However, the evidences of these movements are so abundant and unmistakable that one could not be more certain of them if he had seen the actual flowing of the cell substance.

[1] A portion of this section was published in the *Wood's Holl Biological Lectures for 1898.* (Boston, 1899).

A. Movements during Maturation.

First Maturation.—The cell movements during maturation result in the segregation of yolk and cytoplasm at opposite poles of the egg, and in the transportation of the mitotic figure to the animal pole. While the germinal vesicle is still intact it lies some distance from the periphery and is closely surrounded by yolk spherules, and there is a very incomplete separation of yolk and cytoplasm throughout the entire egg. As soon as the mitotic spindle is formed and the nuclear membrane is broken, there is an area immediately surrounding the spindle and asters free from yolk, but elsewhere in the egg there is an intimate mingling of yolk and cytoplasm. The initial position of the spindle differs in different eggs; it rarely lies in the chief axis of the egg and may be at right angles to this. Gradually the spindle turns until its axis nearly coincides with that of the egg, and at the same time the whole spindle is moved out toward the surface, until finally the outer end of the spindle comes into contact with the cell membrane, and the surface of the egg is elevated into a papilla at this point. This movement is in part due to the mere lengthening of the nuclear spindle which doubles in length during the process, but in part also to a general movement of the cell substance by which the spindle is turned and carried bodily toward the surface of the egg. At the same time there are movements within the egg which lead to an accumulation of cytoplasm at the animal pole and a movement of the yolk spherules toward the opposite pole. There is no evidence that this movement is due to activity on the part of the nucleus and centrosomes. The initial position of the centrosomes and the direction of the central spindle are not the same in different eggs, and yet the final position of the mitotic figure is the same in all cases; the centrosomes and asters at the two poles are identical in form, size and staining reactions until the outer pole of the spindle comes into contact with the surface of the egg. When this occurs the sphere and centrosome at the outer pole become flattened against the egg membrane (Pl. I, figs. 14, 15). At the same time the spindle begins to shorten, and this continues until it is not more than half as long as in the metaphase or early anaphase. At the same time the chromosomes at the outer pole are crowded into the sphere (fig. 16), and finally they are pushed through this until they come into contact with the opposite cell wall (figs. 22, 23). Such a phenomenon is found only in the formation of the polar bodies and must be caused by factors which are wholly different from those which have commonly been held to be active in the mechanics of mitosis. Neither the contraction of mantle fibres (Van Beneden, Boveri), nor the growth of central spindle fibres (Drüner), nor the chemotropic influence of the centrosome and sphere (Strasburger) will explain the extreme movement of the chromosomes in the first maturation. On the other hand the evidence is conclusive that this extreme movement of the chromosomes is due to the same factor which forces the entire spindle into contact with the egg membrane and then causes the flattening of the sphere and centrosome and the shortening of the spindle. Evidently this factor lies wholly outside of the spindle, since it acts upon the spindle as a whole, and must consist of stresses

in the cytoplasm, probably of active movements, and the fact that throughout the egg the separation of yolk and cytoplasm is going on at this time is additional evidence in favor of such general movements in the cell body.[1]

Second Maturation.—The initial position of the second maturation spindle varies greatly in different eggs; in some cases it is almost at right angles to the egg axis (*e.g.*, fig. 28), but it always turns so that one pole lies almost in contact with the mid-body between the egg and the first polar body. It is very probable that the spindle turns into the line of least resistance, and the fact that the line of least resistance leads directly from the chromosomes to the mid-body may be due to the persistence in this axis of the spindle remnants of the first maturation. The second polar body is extruded immediately under the first, so that the latter is raised upon the former and is separated from the egg. This also must be due to the fact that the surface tension is least at this point. Whether this is the result of the fact that the egg membrane is here lifted by the first polar body, or that the surface layer between the egg and the first polar body is here newly formed, or that the cell membrane grows most rapidly over the pole of the spindle cannot be determined by observation alone.

B. Movements during Fertilization.

During the fertilization similar movements of the egg contents are apparently taking place; the polar segregation of yolk and cytoplasm goes on during the approach of the germ nuclei, and, as during maturation, appears to be due to movements of the cytoplasm. The spermatozoon usually enters near the vegetal pole, and is carried through almost the whole diameter of the egg to the animal pole, but it may enter at any place except the protoplasmic area immediately around the animal pole. If the sperm enters at the vegetal pole, its course toward the animal pole is nearly straight; if it enters elsewhere, its course is curved, and the nearer the point of entry to the animal pole the greater the curvature.

The egg nucleus and aster lie very near the animal pole and do not move from this position; they are surrounded by an area of protoplasm free from yolk. The sperm nucleus and aster in their advance through the yolk leave no path behind them; either they are carried along by a general movement of the cell contents, or the yolk is pushed out of their way, to close in again behind them immediately after they have passed. The germ nuclei and asters approach each other, and when the two are close together they lie in an area entirely free from yolk, except that a few spherules are usually found between the two nuclei or asters. These spherules, which are separated from all the rest of the yolk, appear to have been carried before the sperm elements in their advance. After the origin of the cleavage centrosomes the remnants of the asters are carried to a point above the nuclei and immediately under the polar bodies, where they disintegrate and are scattered

[1] Kostanecki and Wierzejsky ('96) have observed in *Physa* that the peripheral movement of the spindle, and the separation of the yolk and cytoplasm go hand in hand.

as coarse granules—a process which will be described more fully when we come to consider the cleavage.[1]

What brings the germ nuclei and asters together? In a former paper ('94) I suggested that the nuclei were passively drawn together by the formation, attachment, and contraction of astral rays, and Kostanecki and Wierzejski ('95) afterward advanced this same view. They maintain that astral rays are strongest while the germ nuclei are being brought together and that as soon as this is accomplished the rays are functionless and disappear. Wilson ('96) regards this view as untenable, and concludes that "the nuclei are drawn together by an actual attraction which is neutralized by union, and their movements are not improbably of a chemotactic character." Morgan ('96) also rejects this idea, and I have myself ('99) practically abandoned it. Nevertheless, unless the nuclei are actively locomotive it must still be true that they are brought together by something outside themselves. This something must of necessity be found in the cytoplasm (including the aster), unless the nuclei are able of themselves to move actively. There is every evidence that the nuclei in this, as in most other cases of movement, are passive, and that their movements are brought about by the activity of the cytoplasm.

The migration of the sperm nucleus, like that of the maturation spindles, is accompanied by progressive separation of yolk and cytoplasm, and it is probable that these coincident phenomena have a common cause in general movements of the cytoplasm.

Furthermore, there are certain elements of constancy in the polar differentiation and in the plane of the first cleavage which cannot be attributed to the nuclei, and, so far as I can see, can be due only to definite characters of the cell body. It is the egg cell rather than the nucleus which shows polar differentiation. The sperm nucleus and aster approach the animal pole from various positions; there is great variation in all the positions of the nuclei and asters relative to each other, and yet there is no variation in the plane of the first cleavage which always passes through the point of extrusion of the polar bodies, and in cases where the first cleavage is unequal the mitotic figure is always eccentric to the same degree. Now the first cleavage, as we shall see, is accompanied by extensive rotary movements of the cell contents, and this fact, joined to the evidences of cytoplasmic movement during maturation and fertilization, leads me to believe that definite movements of cell substance exist in the unsegmented egg. The constancy of cleavage in later stages is associated with constancy of movements in the cytoplasm, and it is probable that the same is true of the constancy which precedes cleavage.

[1] A lobe of cytoplasm appears at the vegetal pole just before the germ nuclei meet, fig. 77. It persists during the first and second cleavages, being nearly separated from the egg during each division of the cell body, fig. 80. It never divides with the cleavage of the egg, but always remains attached to one of the daughter cells, and is gradually resorbed into that cell at the close of the cleavage, fig. 81. This lobe is probably homologous with the "yolk lobe" of *Chaetopterus* (Mead), of *Illyonassa* (Crampton), of *Fulgur* (McMurrich), and of the following gasteropods which I have examined; *Urosalpinx, Nassa, Sycotypus* and four species of *Crepidula*. In all the species of *Crepidula* it contains no yolk and is very small; in *Sycotypus* and *Fulgur* it is larger than in *Crepidula* and contains some yolk, but is still relatively small; in *Illyonassa, Nassa* and *Urosalpinx* it is very large and is filled with yolk.

That the movements within the cell substance of the unsegmented egg are, in certain cases at least, of a vortical character is indicated by spiral asters, first described by Mark for *Limax*, and since observed by several investigators in other animals, and also by my observation that the first cleavage in *Crepidula* is a spiral one, being oblique to the right, or dexiotropic, (see p. 80, also Conklin '97).

C. Movements During Cleavage.

It is, however, in the cleavage of the egg that I have found the most unmistakable evidences of definite and orderly movements of the cell contents. These movements occur before, during and after the division of the nucleus, and are thus characteristic of the entire cycle of division. Since the different cleavages differ considerably in the character and extent of these movements it will be necessary to devote some attention to each cleavage.

The entire history of these movements could never be determined by means of sections alone, though these are of great supplementary value, but recourse must be had to preparations of entire eggs. In such eggs, prepared in the manner specified on page 6, the whole course of these movements can be followed with great clearness and the relative positions of spheres, centrosomes, nuclei and mid-bodies can be accurately determined at every stage.

(1). *First Cleavage.*—At the beginning of this cleavage the cytoplasm is well separated from the yolk in the region of the germ nuclei. Above the nuclei and below the polar bodies traces of the egg and sperm spheres may still be seen, figs. 78, 79. As the spindle elongates and the astral radiations extend, the cytoplasmic area first elongates and then the entire egg becomes ellipsoidal.

From the prophase to the anaphase the mitotic figure lies in a cytoplasmic area almost entirely free from yolk and there are few, if any, yolk spheres between the spindle and the polar bodies, figs. 55, 57, 58 (in the preparation from which figs. 57 and 58 were made, the polar bodies were attached as in figs. 59–61, but in order to save space on the plate the upper parts of the figures, showing the polar bodies, were cut off from the drawings). In the late anaphase, however, the yolk spheres are present not only in the superficial layer of protoplasm, but also in a plane running right through the middle of the spindle, figs. 59, 60, 66, 67; in fact the only area free from yolk spheres at this stage is that immediately surrounding each of the asters. This position of the yolk can have been caused only by extensive movements of the cell substance, the yolk being carried up at the periphery toward the animal pole and then down through the middle of the egg in a plane at right angles to the axis of the spindle (*cf.* figs. 55–61, 80, 81). While this movement is most easily seen and is probably strongest in the direction indicated, *i. e.*, up at the periphery toward the animal pole and then down through the plane of the first cleavage, yet the constriction which forms all around the egg (see fig. 80) shows that the movement must be from the whole equatorial periphery toward the spindle axis. However, this constriction is deepest on the side of the animal pole where this movement is most evident.

Until the anaphase of this cleavage the spindle axis is a straight line, figs. 59, 66. In the telophase the mid-body, which marks the middle of the spindle, is carried down toward the vegetal pole, while the centrosomes, spheres and nuclei are moved up nearly to the animal pole, fig. 60. Finally, in the resting stage the centrosomes and spheres lie almost beneath the polar bodies, the nuclei lie just below these, while the mid-body lies a little below the middle of the plane of contact between the two daughter cells, fig. 61. In short, the spindle axis which was a single straight line up to the anaphase, becomes bent on itself in the telophase and in the resting period until its two halves lie close to each other on opposite sides of the new cell wall.

These movements of the structures which lie in the spindle axis are accompanied by general movements of the cell contents in the same direction. Thus the cytoplasm which is at first spread out in the form of a cap at the animal pole, grows deeper in the telophase, and is carried down with the mid-body to the middle of the cleavage plane; at the same time the yolk is carried up at the periphery toward the animal pole, figs. 61, 79, 80, 81.

The movements in the first two cleavage cells are not, however, directly at right angles to the plane of the first cleavage, but viewed from the animal pole they are slightly dexiotropic, as is shown by the fact that the nuclei, spheres, and protoplasmic areas all move in a dexiotropic direction (fig. 82 and text fig. VIII). The remains of the spheres of the first cleavage can be seen, until the anaphase of the second cleavage, lying near the upper surface of the two blastomeres and close to the wall between them, figs. 83 and 84; in this position they gradually fade out into the cytoplasm, until at the close of the second cleavage no trace of them can be seen. The rotation of cell substance indicated in fig. 82 continues until the superficial extent of the protoplasmic area is smaller and its depth greater than is indicated in that figure, and until the new centrosomes have taken their positions at the poles of the greatly inflated nuclei.

Such a change in the position of these parts could be brought about only by a general rotation of the entire cell body. This general rotation precedes, accompanies and follows the movements of the nuclei, centrosomes and spheres (*cf.* figs. 59, 60, 61), and is, in all probability, the cause of these movements.

(2). *Second Cleavage.*—At the close of the first cleavage the centrosomes lie above and on the outer side of the nuclei (*i. e.*, on the side away from the polar bodies), figs. 82, 83. Here the centrosomes elongate and give rise to the daughter centrosomes and central spindles, which stretch across the nuclei in the groove between the two germ halves, fig. 83, text fig. VIII, while the remnants of the spheres move into the furrow close under the polar bodies. In this position the definitive spindles are formed, while the outlines of the vesicular nuclei, still filled with nuclear sap, are visible on the side of the spindle next the polar bodies even as late as the metakinesis, fig. 84. With the exception of the centrosomes and asters the entire mitotic figure is lodged within that portion of the vesicular nucleus farthest removed from the first cleavage plane, and into the spindle, thus located, all the chromo-

somes are drawn, leaving only the darkly staining nuclear sap in the remaining portion of the mother nucleus, fig. 84.

At this time the nuclei and cytoplasmic areas still preserve their dexiotropic positions in each cell, fig. 83, text fig. VIII; with the breaking of the nuclear membrane, however, the spindles and cytoplasmic areas shift into a læotropic position, fig. 84, and at the same time the surface extent of the cytoplasm becomes greater, and the blastomeres, which had become so flattened against each other that they were nearly perfect hemispheres, again become more and more spherical in shape. The spindles, cytoplasm and entire cell then elongate in the direction of the spindle axis; a constriction appears first at the animal pole and then entirely around the periphery in the equator, and the cell divides as in the preceding cleavage.[1]

In the telophase of the second cleavage the nuclei, spheres and centrosomes again move toward the animal pole while the middle of the spindle and the mid-body are carried down and away from the animal pole toward the middle of each cleavage plane, fig. 86. The direction of this rotation is læotropic in the two blastomeres which lie at the higher level (A and C, fig. 86) and dexiotropic in the two lower ones (B and D, fig. 86). This movement continues until the centrosomes and spheres are carried into the inner angles of the cells, immediately below the polar bodies and until the daughter nuclei, which at first lie very near each other on opposite sides of the second cleavage planes, fig. 86, are swung out into the centers of the cytoplasmic areas, fig. 87.

(3) *Third Cleavage.*—The centrosomes and central spindles then appear as in the preceding cleavage, *viz.*: on the upper and outer sides of the nuclei and in the grooves between the germ halves, figs. 70, 71, 88. The spindles do not at first occupy similar positions in the four cell, but are often dexiotropic in the two lower blastomeres (the ones meeting in the polar furrow) and frequently læotropic in the upper ones, fig. 88. As the cleavage advances, however, the spindles are all turned into a dexiotropic direction. All this time the remains of the spheres of the preceding cleavage occupy the central angles of the cells, and when the upper pole of the spindle moves up under them, their substance is spread in the form of a ring with dense periphery and clear center, figs. 87–90 and text figs. XIX–XX. The spindle and cytoplasm, then elongate, as in the preceding cleavages, and although the cell as a whole does not elongate symmetrically, *i. e.*, at the two poles, it does elongate by the formation of a lobe of protoplasm over the upper end of each spindle. This lobe becomes more and more prominent, and into it the upper pole of the spindle moves. Then the equatorial constriction of the cell begins, forming all the

[1] The equatorial constriction sometimes appears as a broad irregular depression in the cytoplasm beneath the polar bodies (fig. 85). At the margins of this depression there may be serrated processes of cytoplasm which project for a short distance over the depression. I have seen this phenomenon in only a few eggs, and do not know whether it is a normal one or not. It reminds one of the threads and "filose processes" observed by G. F. and E. A. Andrews ('97, '98) in various forms of protoplasm. In this same preparation (fig. 85) the cytoplasm adjoining the first cleavage on each side of this depression is elevated into a ridge; it looks as if the formation of the depression and the elevation of the ridge were parts of the same process.

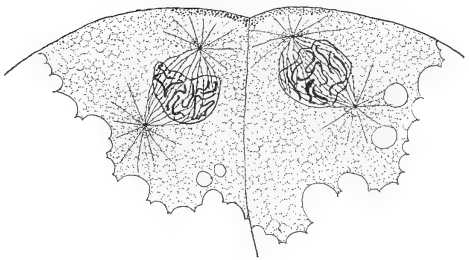

FIG. XIX.

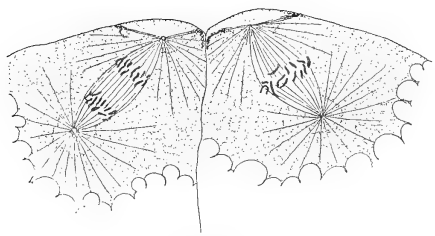

FIG. XX.

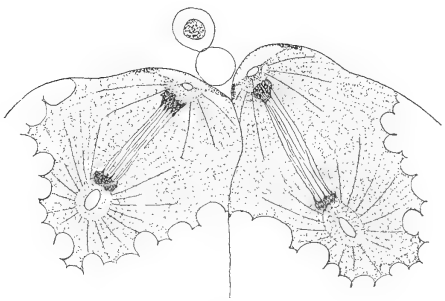

FIG. XXI.

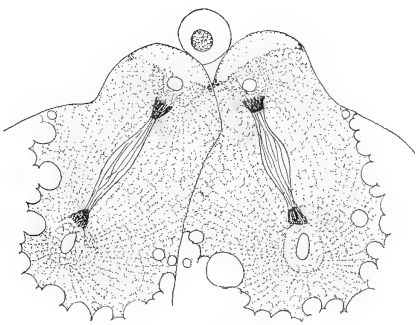

FIG. XXII.

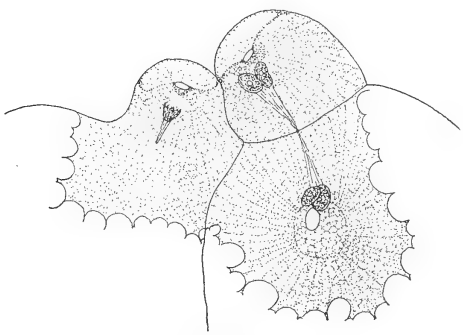

FIG. XXIII.

FIGS. XIX–XXIII.—Successive stages in the third cleavage of *Crepidula*, showing the spreading of the old sphere substance, the lobing of the cytoplasm and the separation of the first quartette of micromeres.

way around the periphery as in the preceding cleavages, and ultimately separating the first group of micromeres from the macromeres, fig. 90 and text figs. XXI–XXIII.

In this division there is a differential distribution of the sphere substance, the whole of the sphere remnants of the preceding division passing into the micromeres while no portion of them goes into the macromeres. During division each cell becomes more nearly spherical than in the resting period, and especially in the late anaphase, when the equatorial constriction is occurring, the daughter cells (both macromeres and micromeres) are so nearly spherical that they touch neighboring cells only by relatively small surfaces, fig. 90. In the telophase and rest they again flatten against one another, fig. 91.

During the telophase of this cleavage the cell contents rotate in a dexiotropic direction in the upper cells (micromeres), and in a læotropic direction in the lower cells (macromeres). Even before the telophase this movement is presaged by the dexiotropic lobing of the cytoplasm in each cell preparatory to the formation of the micromeres; in the telophase it appears in the bending of the spindle axis and in the rotation of the nuclei, centrosomes and spheres. In fig. 90 the earliest bend in the spindle axis is indicated, the middle of the spindle in three of the cells being displaced slightly to the right. Sections through an egg of this stage show that the spindle axes are also bent at the middle toward the surface of the egg, fig. 73. The dexiotropic rotation of the substance of the micromeres continues until the daughter nuclei are carried from the left to the right sides of the cells, though the spheres being in the angles of the cells nearest the animal pole are unable to move through any considerable arc (*cf.* figs. 90, 91). At the same time the substance of the macromeres rotates to the left, until the nuclei, centrosomes, spheres and cytoplasmic areas are carried to the extreme left sides of these cells. Throughout the whole of this movement the centrosomes and spheres never move under the cells of the first quartette, but they always lie on the outer margin of these cells and in contact with a free surface of the macromeres, fig. 91; the nuclei on the other hand are partially or wholly overlaid by the micromeres.

(4). *Fourth Cleavage.*—In this position the centrosomes and central spindles for the fourth cleavage of the macromeres arise from the mother centrosomes, the spindles lying over the upper and outer portions of the nuclei and in the groove between the germ halves as in the preceding cleavages, figs. 74, 75, 91, 92. The initial position of these spindles is very different from their final position; at first their axes are nearly at right angles to planes bisecting each macromere in a radial direction, and the two poles of each spindle are at nearly the same horizontal level. Then the left pole of each spindle rises until it lies immediately under the remnants of the sphere at the surface of the macromere, while the right pole sinks towards the center of each macromere. When the left pole of the spindle approaches the old sphere substance the latter is spread into a ring with dense periphery and clear center, fig. 92, text figs. XXIV, XXV, XXIX, as in the preceding cleavage. Then the cell elongates in the direction of the spindle axis by the formation of a lobe of cytoplasm in the region of the old sphere substance, and at the same time the spin-

dle axis shifts through an angle of about 45°, the upper pole of the spindle being carried in toward the animal pole until the lobe of cytoplasm is pressed into the angle between contiguous cells of the first quartette, fig. 93. In this position the "equatorial" constriction occurs and the second quartette of micromeres is separated from the macromeres.

As the whole of the sphere substance of the second cleavage goes into the first quartette of micromeres, so all the sphere substance of the third cleavage, remaining in the macromeres, goes into the second quartette where it rapidly disappears.

In the telophase the cell contents of the second quartette move in a læotropic direction until the centrosomes and spheres are carried from the extreme left to the extreme right of each cell; at the same time the entire cell contents of the macromeres move in a dexiotropic direction until the nuclei, centrosomes, spheres and

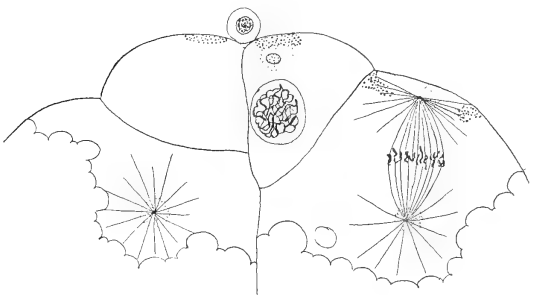

FIG. XXIV.

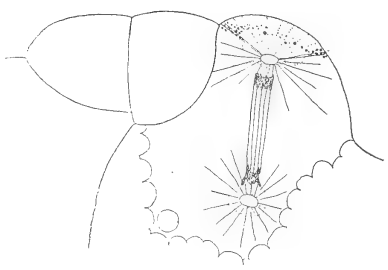

FIG. XXV.

FIGS. XXIV, XXV.—Two stages in fourth cleavage of *Crepidula* showing the spreading of the old sphere substance at the upper pole of the spindle and the lobing of the cytoplasm to form the second quartette of micromeres.

cytoplasmic areas are carried to the right side of each cell (*cf.* figs. 93, 94, 95, text figs. XIII, XIV). As a consequence of these movements in the daughter cells, the spindle axes which were straight lines, until the telophase become bent at the mid-bodies until finally the two halves of each spindle are nearly parallel with each other. This movement of the cell contents and consequent bending of the spindle axes is greater in the second quartette cells than in the macromeres, the rotation in the former being through an angle of more than 90°.

During these movements and throughout the succeeding rest period the centrosomes and spheres are never overlaid by other cells; both in the second quartette and in the macromeres they lie as near as possible to the animal pole without moving under the first quartette cells, fig. 94, 95. If in some cases (*e.g.* fig. 95) they seem to be covered by the cells lying nearer the animal pole, this is due merely to the overarching of these cells, as side views and sections show. In all cases the centrosomes and spheres lie next to free surfaces of the cells.

(5). *Fifth and Sixth Cleavages of the Macromeres.*—While the cell contents of the macromeres are moving from the left to the right side of each cell, the

daughter centrosomes and central spindles again arise from the mother centrosomes and these initial spindles stretch across the outer and upper side of each nucleus in the groove between the germ halves as in the preceding cleavages. The dexiotropic movement of the substance of the macromeres continues until the right pole of each spindle is brought close to the right side of each macromere and into the angle between two adjacent cells of the second quartette. Here the sphere material is spread in a ring as in the preceding cleavages, and a lobe of cytoplasm is formed over the upper pole of each spindle, text fig. XXXI. These lobes are then constricted from the macromeres, thus forming the third quartette of micromeres, fig. 96. Here, as in the preceding cleavages, the whole of the sphere substance left in the macromeres goes into the upper cell products, *i. e.*, in this case, into the third quartette. In the telophase the contents of the third quartette cells rotate in a dexiotropic direction, while those of the macromeres rotate in a læotropic direction (*cf.* figs. 96, 97, 98); the extent of these rotations, however, is not so great as in the preceding cleavages. During these movements in the telophase the centrosomes and spheres never move under the cells lying nearer the animal pole, but always remain at the margin of these cells and in contact with the free surface of the cells in which they lie, figs. 97, 98 and text figs. XV, XVI.

At the sixth cleavage one of the macromeres, D, divides much earlier than the others, and gives rise to the mesentoblast cell, 4d, figs. 97, 98 and text fig. XVI. The centrosomes and central spindle arise from the mother centrosome on the outer and upper side of the nucleus of macromere D, and it is probable that the central spindle lies in the groove between the germ halves, since this groove is plainly apparent in the daughter nuclei at the close of this cleavage (text fig. XVI), showing that the germ halves have been divided, as in all the preceding cleavages. As soon as the nuclear membrane has dissolved at the poles of this spindle the entire macromere becomes rounded and stands out from the other macromeres, being in contact with them by relatively small pressure surfaces. The spindle then becomes nearly vertical in the macromere, its upper pole being inclined slightly to the left. The upper pole of the spindle lies near the surface while the lower pole is near the middle of the macromere. Then a large lobe forms at the upper pole, extending under the micromeres as far as the polar furrow. This lobe contains not only all the cytoplasmic area and sphere substance of the blastomere D, but also a large amount of yolk, text fig. XVI. This lobe then constricts off from the macromere, forming the mesentoblast cell, 4d. This cell is larger than any of the micromeres, and is so covered by them that I have been unable to observe all the movements of its cell contents in the same satisfactory way which is possible in the micromeres. It it certain, however, that its cytoplasmic portion, containing the nucleus, centrosome and sphere, turns in a læotropic direction until these parts are carried under the micromeres and to that part of the cell which lies nearest the animal pole. After this the mesentoblast divides equally into right and left halves as shown in fig. 99, and at the close of this division the nuclei, centrosomes and spheres again rotate through an angle of 90° until they come to lie in those portions

of the daughter cells nearest the animal pole, fig. 100. While these movements are taking place in the mesentoblast and its daughter cells, the substance of macromere D rotates slightly to the right; the nucleus and sphere lie deep in the yolk, by which they are closely surrounded, text fig. XVI. In 4d and its derivatives the spheres are not in contact with a free surface of the cell during the resting period, and in these cells they become very indistinct and can only be clearly recognized during the telophase.

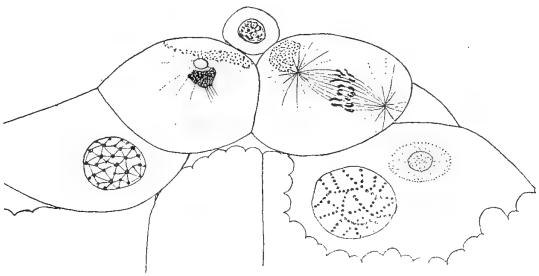

FIG. XXVI.

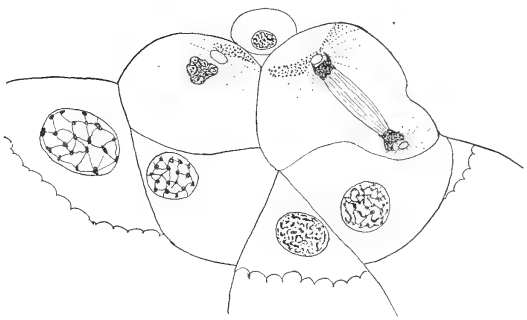

FIG. XXVII.

FIGS. XXVI-XXVII.—Two stages in the first division of the first quartette of *Crepidula*, showing the eccentric position of the spindles and the approaching unequal division of the cells.

(6). *Sub-divisions of the First Quartette.*—During and after the formation of the first quartette, the rotations in these cells are dexiotropic. When the nuclei have been carried from the left to the right side of each cell (figs. 90, 91), they are then moved close into the inner angles of the cells (figs. 92, 93). Immediately over the nuclei are the centrosomes and spheres; the former are already spindle shaped, figs. 91–93, and their long axes nearly coincide with the definitive spindle axes. The central spindles then stretch over the upper sides of the nuclei, very

probably in the groove between the germ halves (*cf.* figs. 76, 92, 93). As soon as the nuclear membrane is broken the entire mitotic figure moves out from the animal pole towards the periphery of the cell; during this movement the sphere substance remains in its former position close to the animal pole. This peripheral movement of the spindle continues until its outer pole comes almost into contact with the cell wall, while the inner pole lies nearer the middle of the cell, fig. 94, text figs. XXVI, XXVII. This position of the spindle is a perfectly definite one; the outer pole of the spindle lies near the middle of the oblique wall between the first and second quartettes, and the spindle axis is parallel with the wall between contiguous cells of the first quartette, figs. 94, 95. Although the spindle nearly doubles in length from the metaphase to the late anaphase, the inner pole remains fixed in position, while the outer pole is pushed further and further into the pointed extremity of the cell, fig. 95. During this elongation of the spindle the cell body also elongates in the same axis, and the equatorial constriction appears and cuts off a small peripheral cell from a large apical one; the small cells are the *trochoblasts*, the large ones the *cephaloblasts*. One of the first quartette cells (1d) is slightly smaller than the other three, fig. 94; it divides a little later than the others, and the cephaloblast to which it gives rise is smaller than the others, though the trochoblasts are all of the same size, fig. 96. This cleavage is a particularly interesting one since it represents a very unequal division of an apparently homogeneous cell. In this case the eccentricity of the spindle would appear to be the immediate cause of the unequal cleavage; this eccentricity is the result of movements in the cell, which begin coincidently with the breaking of the nuclear membrane.

This cleavage is a læotropic one, and in the telophase the contents of the cephaloblasts rotote in a læotropic direction, while those of the trochoblasts rotate in a dexiotropic direction, the middle of the spindle in each cell being carried to the left, text fig. XIV. In the cephaloblasts the nuclei centrosomes and spheres lie nearer the middle of these cells than in the preceding rest stage.

In this position the centrosomes and central spindles for the second division of the first quartette cells arise, as in all the preceding cleavages. The spindles and cell bodies elongate, and the equatorial constrictions occur as is usual. This cleavage is dexiotropic, the spindle axes being at right angles to those of the preceding cleavage (*cf.* figs. 95 and 97). It is also unequal, but in this case the outer products are larger than the inner ones, whereas the reverse was the case in the preceding cleavage. These larger outer products are the *basal* cells in the arms of the ectodermal cross, while the inner ones are the *apical* cells. One of the cephaloblasts, 1d (fig. 97) is smaller than the other three, and its division is not so unequal as that of the others. Accordingly, the basal cell in the arm of the cross in this quadrant, $1d^{1.2}$ (figs. 98, 99), is not so large as the other basal cells; all the apical cells are approximately equal in size. The sphere substance left in the cephaloblasts, at the close of the preceding cleavage, passes at this division into the apicals, where it rapidly disintegrates, fig. 97.

The inequality of this cleavage can scarcely be due to the eccentricity of the

spindles, as a study of fig. 97 will show. The spindles here lie very nearly in the middle of the cells, but the equatorial constrictions show plainly not only that the division will be unequal, but also that the basal cell in the posterior arm of the cross will be smaller than the other three. The cause of the inequality of this division probably lies, not in the position of the spindle, but in the activities of the cytoplasm; and this suggests that the inequality of the preceding cleavage was not caused by the eccentric position of the spindles, but that both the position of the spindles and the inequality of the cleavage are the results of cytoplasmic activities.

In the telophase of this cleavage the nuclei remain near the middle of the daughter cells, but the spheres in the apicals rotate through an angle of from 90° to 180°. In fig. 98 the spheres in these cells are close to the new cell wall between the daughter cells, *i. e.*, they have rotated over the nuclei into this new position, and, therefore, away from the animal pole; that this movement is not merely in a vertical plane is shown by the position of the spheres in fig. 99, where it is evident that the rotation is also dexiotropic, as it should be, since these cells were formed by a dexiotropic cleavage. In the basal cells the spheres move toward the free surface of the cell and a little to the left, figs. 98, 99, but this movement is never extensive; in no case do the spheres move to that portion of the cell which is nearest the animal pole, but they invariably remain on the side of the nucleus farthest removed from that pole. In both of the daughter cells of this cleavage, therefore, the movements are very unusual since the spheres do not move in the telophase as close as possible to the animal pole. The middle of the spindle axis is bent to the right, as it should be following a dexiotropic cleavage (see text fig. XVI).

Especial interest attaches to the cell movements in reversed cleavage or cases in which two successive divisions are in the same direction. Such an instance occurs in the first division of the basal cells of the cross. These cells were formed by dexiotropic division of the cephaloblasts, and to preserve the law of alternation they should divide in a læotropic direction, but they all divide dexiotropically, though the posterior and smaller one does not divide until long after the others (it is still undivided in fig. 100). The reversed cleavage of these cells is associated with the fact that during the preceding rest the centrosomes and spheres remain on the outer sides of the nuclei and do not move to that portion of the cell nearest the animal pole, fig. 99. Therefore the reversal is due to the limited extent of the cell movements, and not to reversed rotations of the cell contents.

At the close of this division of the basals the contents of the upper cells rotate to the right, while those of the lower cells rotate to the left, fig. 100. This is the typical cell movement following a dexiotropic cleavage, and accordingly we may expect to find the subsequent cleavage of these cells entirely typical, an expectation which is fully realized.

(7). *Sub-divisions of the Second Quartette.*—The second quartette cells were formed by a læotropic cleavage, and consequently the rotation within them is in a læotropic direction; this rotation has been fully described on p. 84. When this

lævotropic movement has carried the centrosomes and spheres from the extreme left to the extreme right of each cell, fig. 96, the cleavage begins. The mother centrosome becomes spindle shaped and gives rise to the daughter centrosomes and central spindle. These stretch over the outer and upper sides of the nuclei, most probably in the groove between the germ halves. The right and upper pole of the spindle lies immediately under the old sphere substance, all of which goes into the right daughter cell, where it rapidly disintegrates and disappears, fig. 96, text fig. XXXI. The cell elongates in the spindle axis and the equatorial constriction appears as usual; the division is approximately equal, though the right cell product slightly overlaps the left, and therefore appears larger in surface view, fig. 97.

In the telophase of this division the contents of the daughter cells rotate, as is usual, in opposite directions, the upper or right hand cells to the right, the lower ones to the left. This movement continues until the centrosomes and spheres are carried to that part of each cell nearest the animal pole, and until the mid-body is carried downward and outward between the daughter cells, figs. 97, 98, text fig. XV. This rotation is greater in the left cells than in the right ones, owing to the fact that the latter are partly covered by the basal cells under which the centrosomes and spheres do not move; in the posterior quadrant the basal cell is smaller than the other three and does not overlap the second quartette cells to the same extent, and correspondingly the centrosomes and spheres in the second quartette cells of this quadrant are free to move to the apical side of each nucleus, figs. 97, 98.

In the second division of the second quartette cells the left hand cells divide nearly equally into upper and lower products, and the right hand cells divide very unequally, the upper products being the small *tip* cells of the arms of the cross, figs. 99, 100. The posterior tip cell is larger than the other three, and this is probably associated with the fact that the adjoining basal cell is smaller than in the other quadrants and does not overlap the second quartette cells to the same extent, fig. 100. The divisions in both the right and left cells are lævotropic in direction, and in both the sphere substance of the mother cells passes into the uppermost of the daughter cells. The movements of the contents of these daughter cells in the telokinesis are in all respects typical, *i. e.*, they are lævotropic in the upper products and dexiotropic in the lower ones.

(8). *Sub-division of the Third Quartette.*—The third quartette divides relatively late, and only the first division of these cells will be described here. This division is peculiar, because one of the cells, 3d, divides in a dexiotropic direction, whereas the other three cells of the quadrant divide in a lævotropic direction. Since these cells were formed by a dexiotropic cleavage, the sub-division of 3d in a dexiotropic direction is a violation of the rule of alternation in successive cleavages, *i. e.*, it is a case of reversal. At the time of the division, however, this reversal is slight, the spindle being almost exactly radial; after the division the lower cell moves to the left, so that the position of the daughter cells is such as would result from a very decided dexiotropic cleavage. In the other quadrants the spindles are from the first decidedly lævotropic in direction. The interest in this reversed cleavage is

increased, because it is one of the very first examples of bilateral cleavage in this egg. Owing to this reversal in quadrant D, the position of the third quartette cells in quadrants D and C is bilaterally symmetrical with reference to the plane separating the two mesentoblast cells, fig. 100. If this reversal had not occurred, the position of these cells would have been radially symmetrical, as in the other quadrants and as in all preceding cleavages. The causes of this reversal, therefore, have more than ordinary interest. In the formation of the third quartette the cell contents of 3d, as well as of all the other cells of this quartette, rotate in the telophase in a dexiotropic direction, and to about the same extent in all the cells, figs. 96, 97, 98, text figs. XV, XVI. The cause of this reversal cannot, therefore, be found in the absence or the reversal of the usual cell movements during the preceding telophase and rest. On the other hand, the cap of micromeres in quadrant D is so lifted from the macromeres by the formation of the mesentoblast cell that a space is left between the micromeres and the yolk, and into this space the lower product of the division of 3d pushes, fig. 100. This is not, therefore, so much a case of reversed cleavage as it is one of displacement of daughter cells. Such displacement may occur irrespective of the direction of division or of the movements of cell contents.

Further divisions have been followed in detail up to a late stage in the cleavage, but as they illustrate merely the principles which have been already described, no account is given of them here.

III. Analysis of Movements During Cell Division.

The movements within cells during the cycle of division may be classified under three heads: (1) Movements in Metakinesis, (2) Movements in Telokinesis, (3) Orientation of Centrosomes and Spindles. The first of these has been treated to a limited extent in Part I; however, only those features are there described which are of importance in understanding *nuclear* division; it will now be in order to consider these movements in their relation to the general cell movements.

1. *The Movements in Metakinesis* are of two kinds—movements in the spindle and aster, coincident movements in the cell body. (*a*) *Movements in Spindle and Aster.* As everybody knows the chromosomes, which may be widely scattered through the nuclear cavity, are first drawn into the equatorial plate of the spindle and then separated in the metakinesis, the daughter chromosomes moving toward the poles of the spindle as far as the spheres. Here the movements of the chromosomes cease (except in the single case of the maturation divisions where the chromosomes at the outer pole are pushed right on through the sphere, see pp. 19, 76), and here, in contact with the spheres, the chromosomes become vesicular and fuse to form the daughter nuclei.

The movement of the chromosomes into the equatorial plate is accompanied by a condensation or contraction of the linin network, which is at first uniformly distributed throughout the nucleus (*cf.* text figs. XVII, XVIII, with figs. 57, 65 and 84), and at the same time the greater part of the nuclear sap is squeezed out of this

mass of chromatin and linin into the peripheral portion of the nucleus, and when the nuclear membrane dissolves, into the cell body. The intra-nuclear spindle, although containing a considerable quantity of interfilar substance, is much denser than the surrounding nuclear sap; the radiations of this denser material which surround the equator of the spindle (see p. 18) are apparently due to the fact that in the shrinkage of the linin reticulum some of the fibres of the latter remain attached peripherally, and thus cause a stellate appearance of the spindle when seen in cross sections.

I have already indicated (p. 38) that the cause of the movements of the chromosomes in the metakinesis cannot be found exclusively in the contraction of the mantle fibres, though this may form an important factor; it is probable that this movement is associated with chemotropic attraction between the centrosomes and spheres (Strasburger, Wilson *et al.*). At the time when the chromosomes are being separated the interfilar substance of the spindle aggregates at the two poles, thus contributing to the growth of the spheres. It is probable that this movement is in the nature of diffusion streams, and that the cause of the movement lies primarily in the chemotropic influence of the centrosome. It is scarcely possible that this interfilar substance could be moved by the activity of the spindle fibres, and the fact that the chromosomes move to and partially surround the spheres indicates that their movement may be associated with the same factor which is active in the movement of the interfilar substance.

In the astral radiations the movements are also in the nature of diffusion streams, as was pointed out in the first part of this work (p. 49). In the growth of the aster the denser substance of the alveolar walls (hyaloplasm) is aggregated toward the centrosome, while the more fluid alveolar contents and all cytoplasmic inclusions, such as yolk, are moved farther and farther from the centrosome. The mechanical principles involved in this process have been worked out in detail by Rhumbler ('96). But in addition to this movement there is probably, in the earlier stages of mitosis, a diffusion of nuclear substance from the sphere along the astral radiations. This is indicated by the fact that these radiations stain like the central area of the aster, into which nuclear sap has escaped, and much more deeply than the hyaloplasm of the cell.

In the prophase of the third, fourth and fifth cleavages the upper pole of the spindle lies immediately under the old sphere substance, which at this stage forms a compact, lenticular mass immediately below the cell membrane, figs. 71, 72. In the metaphase this sphere substance is spread for a considerable distance under the cell membrane, its periphery being marked by a thickened ring of this material. As this substance spreads, the rays which go to its periphery remain large and deep-staining, thus forming a kind of "antipodal cone"[1] (Van Beneden), the apex of which lies at the centrosome and its base at the cell wall (text figs. XIX–XXIX). Within this cone the rays are faint and stain little, and the interfilar spaces are

[1] This name is used merely as a convenient descriptive term and without intending to homologize the structure observed by Van Beneden with the one here described. Rhumbler (1901) has called a similar structure in nematode eggs the "Polfontaine."

filled with a clear, non-staining substance. The regular spreading of this old sphere substance over the pole of the spindle is an actual demonstration of Bütschli's ('92, 1900) view that the poles of the spindle represent diffusion centers, from which substances spread over the surface of the cell. There is no conclusive evidence, however, that this diffusion consists of centrifugal movements within the astral rays themselves, since the spreading of the old sphere substance might be brought about by centrifugal movements of the substance between the rays or by peripheral movement of the entire spindle; there is actually such a movement of the spindle, as has been described already (p. 83).

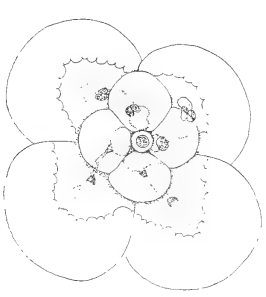

FIG. XXVIII.

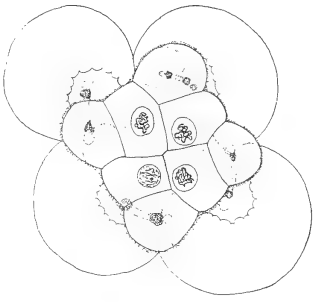

FIG. XXIX.

FIGS. XXVIII, XXIX.—Late stages in the third and fourth cleavages of *Crepidula* showing the spreading of the old sphere substance in the form of a ring at the upper pole of the spindle.

In the late anaphase the sphere substance is again aggregated over the pole of the spindle; this is accompanied by a polar elongation of cytoplasm and the consequent separation of the pole of the spindle from the cell wall, together with the reduction of the angle of the "antipodal cone" mentioned above; at the same time the old sphere material is drawn in toward the centrosome, frequently in the form of a funnel, until its remnants lie close over the centrosome and new sphere, text figs. XXII, XXIII. It seems probable that the aggregation of the old sphere substance, the withdrawal of the astral rays and the coincident growth of the daughter spheres are all dependent upon centripetal movements along the rays during the anaphase.

There is good evidence in favor of the view that the astral rays are absorbed directly into the sphere in the later stages of mitosis. Thus the sphere increases in size and becomes rounded in outline as the astral rays diminish, and though the radiating arrangement of the alveoli may persist right through the resting stage (figs. 61, 69), the substance of the rays has largely disappeared when the spheres have reached their maximum size. Furthermore, after the separation of the daughter cells the spheres always become proportional in size to the volume of the cells in which they lie. Now, the first recognizable difference in the structure of unequal daughter cells is found in the size and extent of the astral rays; the

daughter nuclei, centrosomes and spheres are at first absolutely equal in the two cells, but the astral rays are always proportional in quantity to the volume of the daughter cells. Later, as the astral rays disappear, the spheres grow, becoming in the end proportional in size to the volume of these rays. These facts favor the conclusion that the substance of the astral rays flows into the spheres during the later stages of mitosis.

(*b*) *Movements in the Cell Body.*—While these movements are taking place in the spindle and asters other coincident movements are apparent in the cell body, which lead to the elongation of the cell in the spindle axis and to its ultimate constriction at right angles to this axis.

The elongation of the cell in the spindle axis takes place in every division, even though this axis, when fully elongated, may not be as long as the greatest diameter of the cell. This elongation of the cell may be symmetrical at the two poles, as is the case in all equal cleavages, or it may occur chiefly or entirely at one pole, as is true in very unequal divisions.

There are many reasons for believing that this elongation of the cell is due to a flow of cell substance into the polar areas from the equatorial region of the spindle. The most important of these evidences are found in unequal cleavages in which the elongation of the cell takes place chiefly or entirely at one pole. If one considers either of the maturation divisions or the formation of the first, second or third quartettes of *Crepidula,* one perceives that the cell as a whole does not elongate in the direction of the spindle axis until one pole of the spindle has come close to the cell membrane. After the sphere, and in the case of the maturation divisions, the centrosomes also, have been flattened against the cell wall, the latter protrudes from the general outline of the cell, and into this protrusion the sphere substance, the pole of the spindle and some of the cell substance passes. The elongation of the cell in this case is brought about by this protrusion at one pole of the spindle, and a study of the steps by which this is accomplished shows that there is (1) the movement within the cell which carries the spindle to a peripheral position and presses one pole against the wall, (2) a rapid growth of the cell wall over the pole of the spindle, especially in the area where the sphere is pressed against the wall, (3) a consequent diminution of surface tension at this point and a movement of the pole of the spindle and the cell substance into the protrusion thus formed. If the peripheral movement of the spindle is strong, it may be thrust into this protrusion as far as possible, as in the case of the maturation divisions; if it is less strong the growth of the cell wall and the outflow of cell substance may outrun the movement of the spindle, as is the case in the formation of the first three quartettes. Successive steps in the elongation of the cell preparatory to the separation of the quartettes are shown in text figs. XIX–XXV. It will be seen by these figures that during the prophase and metaphase the peripheral centrosome lies close to the cell membrane, and that the aster is pressed against the cell membrane in the form of a cone, the base and periphery of which are formed of the old sphere substance (sphere remnants of a previous cell cycle). In the metaphase of the third cleavage

this cone has an angle of about 130°. The cell membrane[1] begins to protrude over the base of this cone, the interior of which is filled with a clear non-staining substance; as the cell membrane protrudes, the space between it and the centrosome increases, for although the pole of the spindle moves into this protrusion, it moves more slowly and to a less extent than does the cell substance. During this period of protrusion the base of the cone withdraws from the cell membrane, and at the same time its angle decreases until finally it ceases to touch the membrane and becomes an irregular sphere, fig. 73.

That a similar elongation is taking place at the deeper pole of the spindle is shown by the facts: (1) that a protrusion of cytoplasm surrounding this pole is thrust down into the yolk, which, at the same time, is moved out of the axis of the spindle and up at the sides toward its equator, text figs. XIX–XXIII; (2) in abnormal eggs it frequently happens that a protrusion of the cell membrane takes place opposite the deeper pole of the spindle, as well as at its apical pole, only in this case the protrusion at the lower pole is filled with yolk and frequently ruptures the egg membrane altogether, text figs. XXX, XXXI. This protrusion at the lower pole, in connection with that at the upper one, shows that the surface tension is lessened at points on the cell membrane opposite the poles of the spindle, and further that this is associated with movements of the cell substance from the equatorial to the polar areas of the spindle.

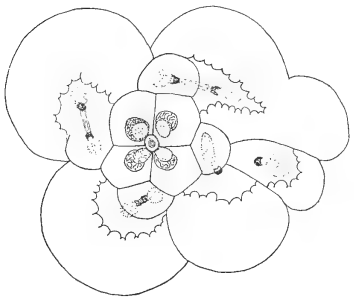

Fig. XXX.

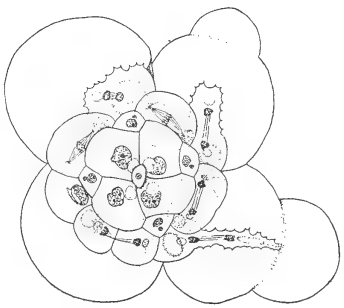

Fig. XXXI.

Figs. XXX, XXXI.—Abnormal stages in the formation of the second and third quartettes of *Crepidula*, showing a lobe of cell substance at both poles of certain spindles.

But while this polar flow of cell substance usually takes place in the spindle axis, it does not follow that the spindle or any part of it is the cause of the flow. On the other hand it sometimes happens in abnormal cleavages that the spindle does not move into its proper position, but remains in the area of one of the future daughter cells; in such cases the cell elongates in its usual direction, but not in the spindle axis, and the constriction and division of the cell body may be wholly typical

[1] In gasteropod eggs the cell membrane is merely a denser layer of protoplasm.

and wholly independent of the spindle, so that in the case mentioned one of the daughter cells would contain no part of the nucleus or spindle. Furthermore, the elongation of the cell usually takes place independently of the spindle in normal cleavages, for here the lobing of the cell is sometimes typically present at a time when the initial spindle may lie in any direction in the cell (*cf.* text figs. XXXII, XXXIII) ; only later is the spindle turned into the longest axis of the cell. We may conclude, therefore, that the elongation of the spindle is not the cause of the elongation of the cytoplasm, but that the latter is quite independent of the former.

The formation of these lobes of protoplasm in unequal cell division strongly resembles the formation of pseudopodia in amœba-like organisms. In the former, as in the latter, there is an outflow of cell substance due to the diminution of surface tension at certain points. In all unequal cleavages the reduction of surface tension takes place principally at one pole of the spindle, and in most of these cases this pole is directed toward the sphere substance of the preceding cell cycle. This old sphere substance lies at the free surface of the cell, immediately under the cell membrane. Here it is spread by the action of the aster, and its granular material probably contributes to the clear plasma of the "antipodal cone" and to the surface layer or cell membrane. A protrusion of cell substance occurs at this place, and into this protrusion the pole of the spindle moves.

The transformation of sphere substance, which is partly derived from the nucleus, into the surface layer or cell membrane affords an explanation of the diminution of surface tension at the poles of the spindle and the consequent elongation of the cell. It also affords a partial explanation of the greater diminution of surface tension at one pole than at the other and the consequent inequality of the resulting cleavage. It is not, however, a full and satisfactory explanation of unequal cleavage, as will be shown later.

In the formation of pseudopodia in amœba-like organisms Verworn ('92, '95) considers that "it is the chemical affinity of certain parts of the protoplasm for oxygen which leads to the reduction of the surface tension at definite places and so to pseudopod formation." How fully my observations on the eggs of gasteropods agree with these conclusions of Verworn will be apparent when it is recalled that sphere substance always moves to a free surface of the cell where it undergoes oxydation, and that in the end it probably takes part in the formation of the cell membrane or the surface layer of the cell.

The Equatorial Constriction.—The evidence which I have just adduced for movements of the cell substance from the equatorial to the polar regions bears also upon the equatorial constriction, since the withdrawal of substance from the equator and its aggregation at the poles must necessarily reduce the equatorial diameter.

Bütschli (1900) has, with characteristic insight, pointed out the fact that if the cell is of a fluid or semi-fluid consistency, the equatorial constriction must be the result of increased surface tension at the equator; and he concludes that the diffusion at the poles and the vortical movements within the plasma can have their origin either in increased tension at the equator or in diminished tension at the

poles. Rhumbler ('97, '99) has emphasized the important fact that in cytodieresis the total surface layer is increased, and that there must be a corresponding growth of the cell wall; he considers that this growth occurs principally in the equatorial plane. In egg cells, I am convinced that the growth of cell membrane takes place principally at the poles, and that the equatorial constriction is due, as Bütschli holds, to relatively greater tension at the equator, this being due to the growth of the membrane at the poles and the consequent decrease of tension at these points. In favor of this view are not only the observations which I have made as to the growth of membrane at the poles, but also the fact that in cells with pigmented surface layer the pigment moves away from the poles of the spindle, while the pigmented cell wall is carried down into the equatorial constriction and ultimately almost all the way through the division plane (see observations of Nusbaum, Van Bambeke and Rhumbler, mentioned later in section on movements in telokinesis). In such cases, therefore, the so-called "new cell wall" is largely the old cell wall, while the new wall is formed chiefly at the poles. However, the central portion of the division wall, *i. e.*, the portion lying near the mid-body, is a new formation.

While the factors just described explain the equatorial constriction, the complete separation of daughter cells and the constriction of the connective fibres of the spindle are probably due to other additional factors. I believe that the principal additional factor in this constriction may be found in the flow of substance from the poles to the equator, near the surface of the cell, and thence into the spindle axis. As evidence of such a flow, I adduce the movements of the yolk spherules in all the divisions of the macromeres, and particularly in the first and second cleavages (see pp. 79 and 81). In all these cases the poles of the spindle, surrounded by cytoplasm, move away from the equatorial plane, and at the same time yolk spherules at the periphery of the cell move toward the equator, and thence in toward the middle of the cell in the plane of the future cell wall. Such a movement is a true vortex, and it might be expected that the yolk spherules which are carried in along the plane of the approaching cleavage would then be carried out through the spindle axis to points on the surface opposite the poles of the spindle. Such, however, is not the case; the yolk spherules lie in the plane of the future cell wall, but never move out through the spindle axis. This cannot be held to demonstrate that there is no movement through the spindle axis toward the poles since the same forces which crowd the yolk spherules and large alveoles out from the centrosomes (see p. 91) would operate to prevent the flow of these large structures through the spindle axis toward the poles.

In all cells, whether they possess yolk or not, it is readily seen that during and after the metakinesis the cytoplasm in the equatorial region stains more faintly, and is composed of larger alveoles as division advances. In fact, the earliest indication in the cytoplasm of the plane where the equatorial constriction will occur is the clear, non-staining zone which runs through the cell in the plane of the future cell wall; this zone is composed of large alveoles with a relatively large amount of non-staining enchylemma and a small quantity of the stainable hyaloplasm. Soon after

the appearance of this clear zone the cell body begins to constrict in this plane, and there can be little doubt that the equatorial constriction is in part the result of the structure of this zone.

The equatorial constriction is, therefore, the result of at least three factors: (1) the decrease of surface tension at the poles and the consequent increase of surface tension at the equator; (2) the vortical flow of cell substance from the periphery of the cell into the spindle axis in the equatorial plane; (3) the structure of the cytoplasm in the equatorial plane, which is here composed of large alveoles with relatively large amount of enchylemma and small amount of hyaloplasm. It is probable that these different factors are all the expression of some common cause, which may possibly be found in the movements of the cytoplasm.

Comparisons.

It is interesting to note how recent work on the movements of cell contents was anticipated by some of the earliest writers on the subject of cell division—I refer especially to Auerbach ('74), Bütschli ('75, '76), O. Hertwig ('75, '77), Strasburger ('75), Fol ('75, '79), Whitman ('78, '87), Mark ('81), *et al.* All of these did their work, at least in part, on living cells, and it is instructive to contrast the plastic, kinetic conception of the cell which they all hold with the rigid, static one which has grown up in recent years, with the development of microscopical technique and the exclusive study of fixed material. Space will not here permit a review of the work of these founders of cytology on the subject of cytokinesis. References to several of these older works are found elsewhere in this paper and an excellent critical review of them may be found in Mark ('81).

To Bütschli, more than to any one else, we owe not only the conception of the structure of protoplasm which is here maintained, but also a mechanical theory of cell division which is built upon the movements of the protoplasm. As long ago as 1876 he considered the asters as diffusion centers (*cf.* views of Auerbach and Bütschli, p. 49), which could increase the surface tension at the equator, while reducing it at the poles; and although now and since 1892 he maintains that the asters exert an attractive influence on the cytoplasm he considers that this attraction (*Zugwirkung*) is still a factor in the constriction of the cell body.

Of all the observations which have heretofore been made on movements during metakinesis those of v. Erlanger ('97[2]) most resemble my own. In the living eggs of some small nematodes he observed strong movements in the cytoplasm in the maturation, fertilization and cleavage. The egg nucleus moves to the center of the egg, apparently by means of plasma streaming, and the approach of the germ nuclei is accomplished by such streaming. During the first and second cleavages the egg plasma shows decided streaming which sets the spindles into slow oscillating motion. The direction of this streaming is from the spindle poles toward the equator on the surface of the egg; at the equator it turns in at the furrow and returns to the poles through the interior of the egg. At the same time the astral rays are bent toward the equator, which still further confirms the existence of superficial

currents from the poles to the equator. These movements in the cytoplasm, though slow and weak, were actually observed by Erlanger in the living eggs. Bütschli (1900) suggests that the cause of this slowness of movement is the viscosity of the cytoplasm, "though powerful, turbulent vortical movements can have no part in normal cell division." The cause of these movements Bütschli finds either in increased surface tension at the equator, or in decreased tension at the poles.

A similar view as to the mechanics of cytodieresis was briefly expressed by Loeb ('95). He suggested that a mechanical explanation of the division of an egg or embryo was to be found in diffusion and vortex movements of the protoplasm, similar to those observed by Quincke in an emulsion of oil and soda solution. "I conceive," says he "that on the surface of the egg, possibly in the meridian or circle whose plane separates from one another the two radiating systems of the centrosomes, diffusion phenomena occur as soon as the nuclear division has physically ended. These lead to the formation of vortical movements, symmetrical in relation to this plane." If these movements are violent they lead to the complete separation of the daughter cells; if not, ordinary cleavage results. It will be observed that in two respects this view of Loeb's differs from Bütschli's and Erlanger's and from my own observations, *viz.*: (1) the diffusion phenomena are not limited to the equatorial circle, and (2) they occur before the nuclear division is ended. Later, Loeb ('95) observed in the segmenting eggs of *Ctenolabrus* droplets over the surface of the egg which collected in the plane of the next succeeding cleavage; this phenomenon he considered a confirmation of this theory.

The movements which occur during karyokinesis in *Crepidula* and other gasteropods entirely confirm the theories of Bütschli and Erlanger as to the mechanics of cell division. These theories also find confirmation in many other observations on a large number of animals. Among these may be mentioned the following:

Morgan ('93) observed that the reddish pigment granules found over the surface of the eggs of *Arbacia* move entirely away from the micromere pole of the egg before the micromeres are formed. In some eggs this movement begins in the two-cell stage, and is carried on until the micromeres are formed at the sixteen-cell stage. Nusbaum ('93) observed in the division of entoderm and mesoderm cells of young embryos of *Rana temporaria* that the brown-black pigment collected in a ring around the equator of the dividing cell, and as the division advanced the ring became narrower and deeper until it formed a true cell plate between the daughter cells. Van Bambeke ('96) has observed a similar phenomenon in the cleavage of the toad's egg. Gardiner ('95) observed in the eggs of *Polychœrus* and *Aphanostoma* a reddish yellow pigment, which, because of its form and peculiar movements, he supposed might be some form of alga. After the egg is laid it migrates from the center toward the periphery, and forms a girdle around the ovum in the plane of the first cleavage. A similar line of pigment marks out the division plane of every succeeding cleavage up to the ten-cell stage. He also observed that these pigment granules migrated from one pole of the egg to the other, though they never passed from one cell to the other. These movements greatly impressed Gardiner with the

wonderfully active and powerful forces within the egg. When the living egg is seen under an immersion lens, he says, "the surface fairly scintillates with the movements of the protoplasm and these pigment granules."

Fischel ('99) has observed a regular and orderly movement of granules, which stain with neutral red, in the living eggs of echinoderms. These granules, which are uniformly distributed throughout the cell during the rest, stream in toward the nucleus at the beginning of division and surround the division figure; after the division they are again distributed throughout the cell. In this case there is no accumulation of these granules in the plane of the cleavage; on the contrary, they move away from this plane. Rhumbler ('96, '99) has studied the movement of the pigment granules of amphibian eggs during division and finds that during the growth of the nucleus they collect around it; in the cytodieresis they are found in the plane of the division wall, and in all cases aggregations of the pigment are found only in thickenings of the hyaloplasm. Van der Stricht ('99) has observed in *Thysanozoon* that at the moment when the nuclear membrane disappears, fatty granules which were scattered through the cell accumulate around the achromatic figure, most of them being found at the equator of the spindle.

Rhumbler ('96, '97) has developed an extensive theory as to the mechanics of cell division. He holds that the spheres do not enlarge by the reception of nuclear substances (as Auerbach, Bütschli, Ziegler and I maintain), but that the nuclear sap is pressed out of the nucleus into the equatorial region of the cell, and that this nuclear sap goes to form the new cell membrane, the amount of membrane formed being proportional to the quantity of sap which escapes ('97, p. 697). I have elsewhere (p. 48) shown reason for believing that the nuclear sap first escapes at the poles of the nucleus and while a considerable portion of the sap may later escape in in the equatorial region, the assumption that the new cell membrane is formed by this sap and that the amount of membrane formed is proportional to the quantity of sap which escapes has little in its favor. Even though some of the sap may escape in the equatorial region it does not always lie where the division wall will form. In the first maturation division the new cell wall forms a considerable distance from the place where the nuclear sap escapes; and in the first and second cleavages the division wall appears all around the equatorial circle, though the sap escapes from the nucleus only in the cytoplasmic area near the animal pole. There is absolutely no reason for believing that in these divisions the nuclear sap collects all around the cell in the equatorial plane as it must do if the new division wall is formed from it. Moreover, the quantity of sap which escapes is not always proportional to the amount of membrane formed. In the case of the formation of the first polar body a larger amount of nuclear sap escapes than at any other mitosis in the whole course of development, yet the increase in the membrane is here perhaps less than in any other cell division, except the second maturation. Therefore, while recognizing the great value and suggestiveness of many of Rhumbler's conclusions, I cannot accept his views as to the formation of the division wall. In egg cells this wall is, as I have maintained elsewhere (p. 96), principally composed of old cell wall infolded at the equator, while the new wall is chiefly formed at the poles.

Rhumbler attributes the phenomena of cytodieresis to at least five factors: — (1) The pull of the astral rays, (2) the pull of the central spindle, (3) the rounding of the cell, (4) the growth of the membrane, (5) the decrease of the nuclear lumen. According to my view the most important factors of all are omitted from this category, viz.: (1) the decrease of surface tension at the poles and the consequent elongation of the cell in the spindle axis, and (2) the vortical flow of cell substance.

2. *Movements during Telokinesis.*—The cell movements during telokinesis are of a rotary character, the spindle axis and cell contents in each daughter cell moving through an angle varying from 30° to 180°. In my former paper ('99) on these movements I did not sufficiently distinguish between the vortical movements of karyokinesis and the rotary ones of telokinesis. There is abundant evidence, however, that the movements of telokinesis are in the main of a rotary and not of a vortical character. The halves of the spindle shift their positions so that they come to lie close to each other on opposite sides of the new cell wall; in general, there is no flow of one portion of the cell contents through another, but all parts rotate in a given plane around some point which serves as a center. In the first and second cleavages the center of rotation is approximately the center of each daughter cell. In the later cleavages the center of rotation varies in different cell generations, but is usually above the center of the cell, *i. e.*, nearer the animal pole, in the case of the micromeres and below the center or nearer the vegetal pole in the case of the macromeres.

(1.) In this rotation the entire cell contents take part; there is not merely a bending of the spindle axis but also a movement of the cytoplasm and yolk. During this rotation the half of the spindle axis in each daughter cell is preserved as a straight line, the bend in this axis occurring only at the mid-body. Throughout the telokinesis the spindle fibres may be recognized connecting the mid-body and nucleus and in some cases passing around the nucleus to the centrosome and sphere, figs. 61, 73. The bending of the spindle axis on itself in the two daughter cells, rather than its rotation with the cytoplasm and yolk, is thus explained by the persistence of the spindle fibres, which attach the structures of the spindle axis to the mid-body. Throughout this rotation the nucleus preserves its polarity, its grooved side (central pole) being turned toward the centrosome, though this general rule may be departed from to a limited extent in cases where the movements of the nucleus or centrosome are interfered with. In the cytoplasm, radiating rows of alveoles are present during the whole of this rotation; in the first and second cleavages they become curved, as shown in figs. 61 and 69. They are entirely lacking on the side of the spindle axis next the new cell wall, where the cytoplasm is clear, non-stainable and shows no traces of alveolar structure.

(2.) The movements in the two daughter cells are always in opposite directions and are always toward the animal pole; consequently, if the rotation is dexiotropic in one cell it is laeotropic in the other. In all spiral cleavages the movements in the upper cell are in the direction of the cleavage by which that cell was formed; thus the first quartette is formed by a dexiotropic cleavage, and the rota-

tion in these cells is dexiotropic, the second quartette is formed by læotropic cleavage and the rotation in these cells is læotropic, etc.

(3). The extent of the rotation differs somewhat in different cell divisions and for different cell constituents, but in all cases there is an evident tendency to carry the poles of the spindle axis to that portion of each daughter cell which lies nearest the animal pole, though this movement is limited by the fact that the spheres do not move away from a free surface and under other cells. Normally the nuclei lie close to the centrosomes and although they may move into that portion of the cell which is overlapped by other cells, they do not separate from the centrosomes; hence it may be concluded that their movements are indirectly limited by this tendency of the spheres to keep in contact with a free surface of the cell.

(4). As a result of the fact that the spheres do not move under overlapping cells, but lie close to a free surface, the centrosomes, nuclei and cytoplasmic areas of the macromeres move down over the periphery of these cells as the cap of ectoblast cells extends until finally they are carried clear around to the vegetal pole. In this way the polarity of these cells is apparently reversed, the nuclei, centrosomes and cytoplasmic areas being carried from the animal to the vegetal pole, in front of the margin of overgrowing ectoblast cells. Another result of the fact that the centrosomes and spheres lie in contact with a free surface of the cell is that the cells are formed in a one layered epithelium and not in a many layered one or in a solid mass. Cells are not budded off from the macromeres under the cap of ectoderm cells but at its edge, and in the subdivision of the ectoderm cells the same principle is operative; thus although the ectoderm cells may overlap one another to a certain extent they are never completely covered by other cells but always preserve a free surface. Heidenhain ('94) has shown that in one layered intestinal epithelium the centrosomes during the rest lie between the nucleus and the free surface of the cell; in division the centrosomes lie 90° from this position, the spindle being paratangential with the free surface of the cell; he has pointed out the fact that if the angle of rotation of the centrosome were different an entirely different form of cell complex might result. In *Crepidula* it is not the angle of rotation which determines that the ectoderm shall form a one-layered epithelium, since this angle varies with every cleavage, but the fact that in the rest the centrosomes and spheres lie next a free surface of the cell.

In the formation of the mesentoblast (4d), however, there is an important exception to this general rule. In the late anaphase of this cleavage the centrosome and sphere are still in contact with a free surface of the cell 4d, (text fig. XVI) but in the telophase the nuclei, centrosomes, spheres and cytoplasmic areas are carried under the overlying ectoderm cells, only that portion of the cell which contains yolk remaining at the surface. It is difficult to observe the centrosomes and spheres in the cells derived from 4d, but during the first two or three cleavages they lie on the apical, *i. e.*, animal pole, side of the nuclei during the resting period, fig. 100. In all these derivatives of 4d the spheres stain less densely and are larger and less definite in outline than in those cells in which they are in contact with a free surface. I

have been unable to determine why the centrosomes and spheres in this single case move under other cells and thus give rise to a middle layer.

(5). Generally these telokinetic movements continue throughout the whole of the period which is commonly called the "rest." They grow less and less evident, however, as the prophase of the next division approaches and, for a brief period before the next cleavage begins, cease altogether. This brief period we may call the "pause" (Fol '96). During the pause the nuclei frequently lie in that portion of the cytoplasm which will form the larger of the two daughter cells at the next division. Thus in the pause preceding the first subdivision of the first quartette the nuclei lie as close as possible to the animal pole, figs. 76, 91, 92, 93, and these portions of the cells become the large cephaloblasts at the following cleavage; in the pause preceding the second division of the first quartette, the nuclei lie some distance from the animal pole in those portions of the cells which will become the large basal cells at the next division, fig. 96, text figs. XIV, XV. This signifies more than that the nucleus lies in the center of its working sphere, since the nucleus does not lie in the center of the cytoplasm, but always in a position which has reference to the future division; the equality or inequality of the division is already predetermined before any trace of that division has appeared.

(6). Finally, the movements in telokinesis are in some way caused by the polarity of the protoplasm of each cell; in fact every blastomere behaves much as does the entire egg before cleavage begins, its substance rotating until the cytoplasm, nucleus, centrosome and sphere are carried to that portion of the cell nearest the animal pole. During the cleavage the spindles lie in many directions and cells are formed in many positions, but after every division the original polarity of each cell is, as far as possible, restored. Further, this rotation may be associated with the movement of the poles of the spindle, through chemotropic influence, to a free surface of the cell. The fact that the spheres become pressed against the cell membrane and that in this position they undergo changes in form and staining reactions, staining more deeply and becoming more coarsely granular, suggests that they here undergo some chemical change, probably an oxydation.[1] This factor, however, will not account for the fact that the spheres move in a predetermined course as near as possible to the animal pole and that the whole cell contents move with them; this movement is evidently reducible to that class of movements which brings about the polarity of the egg, but the causes of these movements I am unable at present to analyze further.

Comparisons.

(*a*) *Protozoa and Protophyta.*—Lauterborn ('96) has observed that the nuclei and centrosomes rotate through an angle of 180° at the close of division in diatoms.

[1] Attempts to determine experimentally whether the spheres move to a free surface under the influence of oxygen have so far been inconclusive, since all movements, as well as other developmental processes cease in the complete absence of oxygen (*e. g.*, in an atmosphere of hydrogen). However in sea water which has been boiled in order to drive off contained gases and then cooled in stoppered tubes, eggs develop irregularly, the micromeres no longer being arranged in a one-layered epithelium over the yolk, but forming irregular heaps and masses, such as would result from the failure of the spheres to move to a free surface.

R. Hertwig ('99) has seen a similar phenomenon in *Actinosphærium*. He says, p. 692, "Ehe die Theilung zu ende geführt ist, wird die Spindelkörper bei der typischen Karyokinese der Actinosphærium über eine Seite gebogen, so das die Tochterkerne später dicht bei einander liegen (taf. III, figs. 10, 11) oder er wird bei dem Richtungstheilungen fast rechtwinkelig eingeknickt (taf. V, figs. 15, 16)."

(*b*) *Tissue Cells and Testis Cells.*—M. Heidenhain ('94) in his great work on the centrosome first described in detail the bending of the spindle axis and the movements of the centrosomes and nuclei at the close of division. These movements he designated "Telokinesis" and he properly recognized that they constitute the final stage of cell division to which he gave the name "Telophase."[1] These movements were observed in leucocytes and one-layered epithelium, and Heidenhain supposed that they might be present in the division of many other cells. According to Heidenhain the movements of the microcenter take place in a curve parallel to the surface of the cell, while the nucleus probably moves in the reverse direction. In one-layered epithelium the axis which passes through the microcenter and nucleus, *i. e.*, the cell axis, moves through an angle of 90° after each division; in embryonic development it moves through varying angles. The result of these movements is to bring the microcenter to the center of the cell and the nucleus to a peripheral position. Heidenhain was unable to determine whether in these movements the nucleus rotates so as to preserve its inner polarity. The cause of these movements he finds in the contraction and expansion (*Spannung*) of the organic radii, *i. e.*, through a lengthening of the polar group of radii and a shortening of the radii which stretch over the nucleus to the opposite side of the cell.

Erlanger ('96) found in the division of the branchial epithelial cells of the salamander that the daughter cells regularly turn through an angle of 90° or more toward the spindle axis of the mother cell.

Similar movements have been observed in testis cells by Meves ('94, '96), Moore ('95), and Prenant ('95). The latter has seen extensive movements of the microcenter in the telophase of the testis cells of *Scolopendra*. The microcenters of the two daughter cells are inversely symmetrical with reference to the axis which joins the nuclei, the one being situated to the right the other to the left of that axis. In some cases, however, the microcenters lie on the same side of the axis, *i. e.*, the symmetry is not inverse. Remnants of the spindle remain as a "perinuclear band," which, he thinks, may be the agent of the movements of the microcenters. He does not agree with Heidenhain that the microcenter lies in the center of the cell during the rest, and this is certainly not true of the mollusks which I have studied.

In the spermatocytes of elasmobranchs Moore finds that the centrosome, surrounded by archoplasm, wanders toward the equator, and when it has reached a point between the pole and the equator it moves to the cell periphery; the centrosome here lies between the chief mass of archoplasm and the cell wall.

[1] The custom of using this term to designate the final stages of the anaphase (*cf.* Wilson '96 and 1900, Coe '99, Griffin '99, *et al*) is to be deprecated, since Heidenhain's definition of this term is perfectly explicit and the stage to which it applies is clearly marked off from the anaphase.

Meves ('96), in the spermatogonia and spermatocytes of the salamander, finds the center close under the cell wall in the anaphase; in the case of the smaller spermatogonia and spermatocytes the centers move from this position through an angle of 45° to 135°; in the larger spermatogonia no lateral movement takes place, but only a movement toward the equator in the spindle axis, which is probably caused by the contraction of the earlier spindle fibres. Meves believes that the lateral movements of the centers in the smaller cells are caused by the development of large and numerous astral rays on the side from which the centers move, which rays serve to push the centers into their new positions. These movements do not take place in a definite direction nor are they in the same plane in the two daughter cells. In the eggs which I have studied this movement cannot be caused, as Meves assumes, by the pushing of polar rays on the side from which the centers move, for in these eggs the whole cell contents rotate, as has been described.

Montgomery ('98) has observed in the testis cells of *Pentatoma* that the new centrosome appears at a point in the cell about 180° from that occupied by the old centrosome. He has also observed that the idiozome material moves from the poles to the equator of the dividing cell.

(*c*) *Ova and Blastomeres.*—Mark ('81) first observed and figured a bent spindle axis in the egg of *Limax* (see his figs. 91 and 93, in which the middle of the spindle is shown displaced toward the center of the egg). MacFarland ('97) has shown the same thing in one of his figures of *Pleurophylidia* (fig. 20), though he figures the centrosomes as lying below the level of the nuclei, a thing which I have never observed in any mollusk.

Kostanecki ('97) says that in the cleavage of *Physa* the daughter cells take opposite positions in the telophase while they turn against the spindle axis through an angle of as much as 90.° The *Zwischenkörper* does not, therefore, lie in the middle of the equatorial constriction, but is shoved to one side.

Quite recently Rhumbler (1901) has described a periodic movement of the nucleus to the cell surface within the living blastomeres of certain nematodes. At the close of each cell division the nuclei migrate to certain places on the cell surface, which places lie in the plane of cleavage of the following cell division. Between the nucleus and cell surface Rhumbler has observed a clear area which he calls the "Polfontaine," and which probably corresponds to the sphere of *Crepidula*. The movements observed by Rhumbler entirely correspond to the movements in telokinesis which I had previously described ('99) though he has evidently overlooked my paper on this subject.

Zur Strassen (1901) also has recently described the position of the centrosome in the resting cells of *Ascaris megalocephala*. In brief, he finds that during the resting period the centrosomes lie close to the cell surface, and he describes in detail the symmetrical movement of the centrosomes and spheres toward the division plane between two daughter cells. This movement in every respect resembles the movements in telokinesis which I have described, and applies to the nuclei as well as to spheres. Zur Strassen further finds that the form of each cell changes with the

movements of the sphere, the cell wall being especially prominent over the sphere. He also discusses in a very suggestive manner the relation of these movements to the morphological and physiological polarity of the cell.

This is doubtless an incomplete list of the cases which have heretofore been observed in which there is a decided bending of the spindle axis at the close of division, but the cases are sufficiently numerous to indicate that this is probably a general phenomenon.

3. *Orientation of Centrosomes and Spindles.*—The centrosome, which during the anaphase is usually spherical, becomes ellipsoidal or spindle-shaped during the telophase and rest. The axis of elongation of the centrosome becomes the initial spindle axis. It is nearly constant in direction for any given cell generation, but differs somewhat in different generations. No general rule can be formulated with regard to the relation of this initial spindle axis to the old spindle axis, or rather the half of it which lies in each daughter cell, but the new axis is most frequently at right angles to the old, figs. 82, 83, 86, 91.

As the initial spindle elongates and the peripheral layer of the old centrosome disappears, the new spindle moves out of the old sphere, which at once becomes irregular in outline; at the same time the new spindle moves over the surface of the nucleus until it comes to lie in the groove separating the germ halves and until the poles of the spindle lie at opposite sides of the nucleus. In some cases the initial spindle lies almost in the groove between the germ halves of the nucleus when first formed (*e. g.*, prophase of the second cleavage, figs. 82, 83, text figs. VII, VIII); in other cases it must move some distance before taking up this position (*e. g.*, earliest stages in the third and later cleavages, figs. 86, 88, 91).

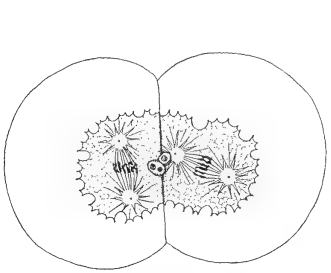

FIG. XXXII.

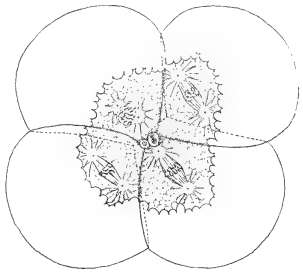

FIG. XXXIII.

FIGS. XXXII, XXXIII.—Metaphase of the second and third cleavages of *Crepidula*, showing an unusual position of certain spindles; these ultimately rotate into normal positions.

The position of the initial spindle in the nuclear groove does not always correspond to its definitive position. In many cases the latter is reached only after more or less extensive movements of the entire mitotic figure (*cf.* fig. 88 and text figs. XXXII and XXXIII). These movements are of two kinds, a rotation of the spindle into its definitive axis and a transportation of the entire figure to its final position in the cell, figs. 93, 94.

The orientation of the centrosome and spindle has reference therefore to two different things, (1) the relation of these structures to the nucleus, (2) their relation to the cell body.

(1). *Orientation of Centrosome and Central Spindle relative to the Nucleus.*—It is evident that some kind of connection exists at all stages of the cell cycle between the centrosome and the nucleus. This is of course most evident during division when the two are connected by the spindle fibres, but even during the rest there must be some connection since the two lie in close contact and except in abnormal cases do not separate. Whether this connection during the rest is in the form of fibres (possibly a persistence of those which previously connected centrosome and chromosomes) or is the expression of some other mechanical action or of a chemotropic attraction does not appear from my studies. The movement of the initial spindle out of the sphere and into the groove between the germ halves of the nucleus must be attributed to some such connection between the centrosome and nucleus. Of course the mere separation of the centrosomes until they lie as far apart as the diameter of the nucleus may be attributed to the growth of the central spindle.

(2). *Orientation of the Mitotic Figure relative to the Cell Body.*—In the early prophase the entire mitotic figure moves in a direction opposite to that of the previous telophase; or, in a word, the prokinesis is the reverse of the preceding telokinesis. For example, in the telophase of the first cleavage the cell contents move in a dexiotropic direction until nuclei, centrosomes and spheres are carried close to the division wall and near to the free surface of the cells, fig. 82; in the following prophase they move away from the division wall, in a læotropic direction and deeper into the cell (*cf.* figs. 82 and 84, also figs. 63 and 65). In the telophase of the second cleavage the daughter nuclei and centrosomes move close to each other on opposite sides of the division wall, fig. 86, and then into the apical angles of the cells, fig. 87; in the following prophase they move farther from the apical pole and a little deeper into the cell, (*cf.* figs. 87 and 88). In the telophase of the third cleavage the nuclei of the macromeres move in a læotropic direction until they come into contact with the wall at the left of these cells, and toward the apical pole until they are almost entirely covered by the overlying micromeres, fig. 91; in the prophase of the next division of these cells the mitotic figures move away from the animal pole and a little to the right, (*cf.* figs. 91 and 92). In the telophase of the third cleavage the nuclei of the micromeres move to the right and as near to the animal pole as possible, fig. 92; in the prophase of the next division of these cells the mitotic figures move away from the animal pole and a little to the left, (*cf.* figs. 93 and 94). The same principle is shown in the first division of the second quartette (*cf.* figs. 95 and 96). In fact it may be laid down as a general rule that the movement in the prophase (prokinesis) is in the reverse direction of that in the telophase (telokinesis), though usually not so extensive.

Other movements of cell contents, which bring about peculiarities in the direction of division and hence peculiar types of cleavage (radial, spiral, bilateral), or

marked eccentricity of the mitotic figure and subsequent inequality of cleavage are intimately associated with processes of differential cell division and the discussion of this subject is postponed to the next section. It need only be said here that there are many evidences that the movement of the entire mitotic figure into its definitive position is a function of the cytoplasm, rather than of the nucleus, centrosome or spindle. This movement does not occur until after the nuclear membrane is dissolved at the poles of the spindle and it is probable therefore that the escape of substance from the nucleus acts as a stimulus to the cytoplasm which then moves and behaves in a predetermined manner.

IV. Some Factors of Differentiation.

In conclusion a brief summary may be given of the bearing of cytokinesis on problems of differentiation. In the main, differentiation in the early development of an animal consists in the formation of various unlike substances and in their definite localization in different regions of the egg or in different blastomeres. In this localization one of the most important principles is polarity.

1. *Polarity:* (*a*) *Unsegmented Egg*.—The polarity of the egg, by which is meant the localization of unlike substances and structures with respect to a single axis, the chief axis of the egg, is indicated before maturation by a slight eccentricity of the germinal vesicle; the latter is, however, entirely surrounded by yolk and the cytoplasm is uniformly distributed throughout the egg. About the time of the entrance of the spermatozoon the wall of the germinal vesicle dissolves and at once movements within the egg substance begin which ultimately lead to the segregation of yolk at one pole and of cytoplasm at the other. There is good evidence that this segregation takes place along preexisting lines of structure, the cytoplasm, mitotic figure and escaped achromatin moving to that pole toward which the germinal vesicle was eccentric, and in all cases this probably corresponds to the free pole of the epithelial cell from which the egg was derived.

In the gasteropods which I have studied this movement is in no way correlated with nor influenced by gravity, the place of entrance of the spermatozoon, nor, so far as I can see, by any other extrinsic factor. The fact that in some animals the yolk has a greater specific weight than protoplasm has led O. Hertwig ('93, p. 215) to assert as a general law that "Polar differentiation consists in this, that the lighter protoplasm collects at one pole and the heavier yolk substance at the other." Rhumbler ('99, p. 568), also says "Incontestibly the yolk granules (in telolecithal eggs) are collected in the lower part of the egg through their greater specific weights." Where the yolk is heavier than the protoplasm this may of course be true, but it is by no means generally applicable. In many well known cases among annelids, mollusks and arthropods gravity has no determining influence on the polarity of the egg which is established in a predetermined axis irrespective of the position of this axis with reference to the direction of gravity.

In the gasteropods both polar bodies are extruded at the same point on the surface of the egg, and the animal pole thus established bears an invariable relation to

all future development. Through this pole the first two cleavage furrows always pass; around it three quartettes of ectomeres are formed, each of which has a definite developmental history and gives rise to definite parts of the larva or adult; certain of these blastomeres are visibly different from each other in size, position, shape and quality, and although these differences arise gradually in the course of development, the polarity of the unsegmented egg exercises a determining influence upon all of them. This polar differentiation of the egg is therefore of the greatest prospective significance.

The eccentricity of the germinal vesicle, which is the earliest evidence of this polarity in the free egg, is itself, most probably, the result of polarity already existent in the cell from which the egg is formed. This polarity must be regarded as the factor which directs the general cell movements which bring about the segregation of yolk and cytoplasm; while the immediate cause of these movements is very probably the escape of achromatic substances from the nucleus.

In the fertilization of the egg the same sorting of the egg contents continues as in the maturation, with the result that at the beginning of the first cleavage almost all of the yolk is collected at the vegetal pole while the greater part of the cytoplasm lies close around the animal pole.

(*b*) *Blastomeres.* Every blastomere manifests the same type of polarity as the unsegmented egg itself. At every cleavage this polarity of the blastomeres is lost or modified, only to be reestablished again in each telophase. In every division of blastomeres containing yolk the mitotic figure surrounded by cytoplasm moves down into the yolk area while the yolk moves up at the periphery toward the animal pole. In the telophase and resting period, however, the centrosomes, nuclei and cytoplasm again take a superficial position near the animal pole, while the yolk again moves toward the vegetal pole. This polar movement concerns not only cytoplasm and yolk but also different kinds of protoplasm. Thus the sphere substance always takes a definite polar position in each blastomere and the localization of certain characteristic kinds of cytoplasm (hyaline or granular) is also referable to polarity.

In each blastomere the cell axis (Heidenhain), by which is meant the line passing through the center of the nucleus and centrosome, shifts during the telophase until the centrosome and sphere are carried to a free surface of the cell and as close as possible to the animal pole. The result is that in all the early cleavages the cell axis tends to become parallel with the original egg axis, though this is prevented in many instances by other cells which lie nearer the animal pole.

(*c*) *Nucleus, Centrosome and Sphere.*—During the rotation of the cell contents in the telophase the spindle axis rotates as a whole so that the nucleus at all stages in this rotation presents approximately the same side (its central pole) toward the centrosome and sphere, and its opposite side (distal pole) toward the mid-body. Throughout this rotation the centrosome and sphere also present the same side toward the nucleus. The polarity therefore of the nucleus, centrosome and sphere, as well as that of the cell body, is reestablished after every division.

The immediate cause of these telokinetic movement, as also of those in the unsegmented egg, may be found at least in part in the movement of the spheres to a free surface of the cell, but the orientation of these movements cannot at present be further explained than to refer them to the structure of the protoplasm.[1]

2. *Differential Cell Division.*—As has been emphasized already (p. 6), cell division is typical and non-differential when it occurrs at regular intervals or at the same time in cells of the same generation (rhythmical), when successive divisions are at right angles (alternating), when the daughter cells are of similar size (equal) and are composed of similar materials (homogeneous). Divisions are differential when they depart from these typical conditions in one or more respects, thus becoming non-rhythmical, non-alternating, unequal or heterogeneous.

(*a*) *Rhythm of Division.*—A definite order of cleavage is highly characteristic of gasteropods. In *Crepidula* one of the first two blastomeres usually divides slightly earlier than the other, and in the formation of the first, second, third and fourth quartettes one cell of each quadrant forms earlier than the other three. These differences in the time of formation of the different cells of a quartette are least in the first quartette and greatest in the fourth, where the cell 4d is formed at the 25-cell stage while the other cells of this quartette are not formed until the 52-cell stage. In the subdivision of the different quartettes this same lack of rhythm is observed, the cells which formed first being usually the earliest to divide. To this rule, however, there are several notable exceptions; for example, the trochoblasts which are formed at the first division of the first quartette do not again divide until more than one hundred cells are present. In this case the lack of rhythm in the divisions leads to important differentiations, since the large trochoblasts give rise to certain of the large cells of the velum and head vesicle.

The old view (Balfour '80) that the rate of division depends upon the presence or absence of yolk, cells with yolk always lagging behind those without it, is untenable since this lack of rhythm frequently concerns purely protoplasmic cells. For example, none of the ectomeres of *Crepidula* contain yolk, yet they divide at very different rates, while on the other hand many yolk-laden cells divide more frequently than those without yolk (see Wilson, 1900, p. 366).

The rhythms of division of centrosome, nucleus and cell body go on more or less independently of one another. Boveri ('97) has shown that in echinoderm eggs the centrosomes preserve their rhythm of division even when separated from their nuclei, and I have observed the same thing in enucleated blastomeres of *Crepidula*. Furthermore, the rhythm of cell and nuclear division are more or less independent of each other; in certain abnormal eggs of *Crepidula* I have observed that normal and characteristic cell division may occur in enucleated blastomeres, and on the other hand nuclear division may go on in regular manner and at regular intervals in the absence of cell division. There is therefore no absolutely necessary connection between the division of nucleus, centrosome and cell body.

Driesch ('98) has shown that in cross-fertilized eggs of echinids the rhythm of

[1] See similar conclusions reached by Lillie (1901) in the case of *Unio*.

cleavage is that of the maternal and not of the paternal species; in this case the rhythm depends upon the egg cell and not upon the sperm and therefore, in all probability, upon the cytoplasm and not upon the nucleus or centrosome.

While the divisions of nucleus, centrosome and cell body may occasionally go on more or less independently, it is certain that they are normally intimately connected, and I believe that the normal rhythm of division is largely determined by the interrelation of these structures. In *Crepidula* it is always possible to determine whether or not a cell will soon divide by the relative size of the nucleus as compared with the cell body. Both nucleus and cytoplasm increase in volume after each division, but the nucleus increases much more rapidly than the cytoplasm.[1] When the nucleus has reached a certain maximum size relative to that of the cell body it enters upon the prophase of the next division. The centrosome sometimes divides and gives rise to the initial spindle before the nuclear prophase and in such cases it seems to wait for the nucleus before going through the further stages of its separation. The cytoplasm also sometimes elongates in the direction of the coming division, but seems to wait for the nuclear prophases before undergoing constriction.

Strasburger ('93) determined the relative size of the nucleus to the cell body in some forty species of plants. He found that while this ratio differed in different species and in different organs of the same species, yet in a given organ of a given species it was quite constant. The average ratio of nuclear to cell diameter in embryonic cells he found to be about as 2:3. When the diameter of the cell, as compared with that of the nucleus, exceeds this ratio, cell division occurs and the ratio is thus restored.

In *Crepidula* it is difficult to establish such a ratio owing to the differences in in the shapes and dimensions of cells in the early cleavage; I have, however, measured a number of cells and nuclei in the prophase of the first maturation and of the first, second and third cleavages, and the ratio of the nuclear to the cell diameter in these yolk laden cells is about 2:7. Such measurements show that at the moment of division there is a fairly definite ratio between the diameter of the nucleus and that of the cell, but whether the growth of the nucleus beyond this ratio is a stimulus to division or is merely an accompaniment of it, is not indicated.

(*b*) *Direction of Division.*—Upon the direction of division depends the relative positions of the daughter cells and consequently the type of cleavage, viz.: radial, spiral, bilateral or teloblastic. In determinate cleavage this is an important factor of differentiation since it leads to the localization of cells and different cell substances.

The direction of cell division has been attributed by various authors to a variety of factors; thus it is said to be due to the fact that the mitotic figure lies in the direction of least resistance (Pflüger), or in the longest axis of the protoplasmic mass (Hertwig); the shape and position of cells and consequently to a certain extent the direction of division, are said to be due to the rectangular intersection of cleavage

[1] Since the egg as a whole does not increase in size until the gastrula stage the increase in the quantity of cytoplasm must be at the expense of the yolk.

planes (Sachs), or to the principle of smallest surfaces (Plateau, Berthold). Almost all investigators agree that the direction of division is due to the position of the mitotic figure and the orientation of the figure is usually supposed to be actively produced by the figure itself, or by some part of it. According to Roux ('95) there is immanent in the nucleus, a direction of division which may be independent of the chief dimensions of the protoplasmic body. Rauber ('83) holds that the position of thespindle is the result of the mutual attractions of neighboring asters. Heidenhain ('94) refers the direction of division to a definite angle of rotation of the centrosomes.

In the segmenting eggs of the gasteropods which I have studied the direction of division is not primarily due to any of the factors named, though several of these principles are well illustrated in these cells. The spindle usually, though not invariably, lies in the longest axis of the protoplasmic mass and it probably always lies in the direction of least resistance, though this in itself is no explanation of the direction of division. As has already been pointed out (p. 105), the angle of divergence of the centrosomes and the initial position of the mitotic figure may not correspond with its final position, while at the same time the lobing of the cytoplasm may indicate the final position of the spindle and the direction of the coming division; in fact the form of the cell and the movements of the cell contents may proclaim where the next division will occur long before the spindle is formed.

The alternation in the direction of successive cleavages is not due to the mere divergence of the centrosomes in planes successively at right angles to one another, but rather to regular alternations in the rotations of the cell contents; the lack of alternation is associated (at least in one generation of cells, see p. 88) with the lack of rotation of the cell contents during the preceding telophase.

When for any reason the mitotic figure is prevented from assuming its normal position, the cytoplasm may divide in the normal place and manner, thus giving rise to a cell which is normal in appearance except that it contains no part of the nucleus or spindle.[1] From these facts I conclude that the position of the spindle is the result of movements and stresses in the cytoplasm. In normal cell division the spindle takes a position of equilibrium between the two portions of the dividing cell, so that the equatorial constriction cuts through the middle of the spindle; if, however, the spindle is prevented from assuming this position of equilibrium it may be cut through nearer one end than the other or may be left entirely to one side of the new cell wall. Therefore the position of the spindle and the direction of division are functions of the cytoplasm, rather than of the nucleus, centrosome or spindle.

(*c*) *Size of Daughter Cells.*—The inequality of cell division leads to some of the most characteristic and important features of differential cleavage, while the varying sizes of blastomeres have a definite prospective significance in development, as Lillie ('95, '99) and Conklin ('97, '98) have pointed out.

[1] I have seen several such cases, but a more detailed account of them must be postponed to another paper.

The inequality of cell division is most commonly referred to the eccentricity of the mitotic figure, however this may be caused, (see Wilson 1900); but it is sometimes associated with a lack of symmetry in the spindle itself. Thus R. Hertwig ('99) finds in *Actinosphærium* that one pole of the spindle (*Richtungskörperpol*) differs decidedly from the opposite pole, and the new cell wall cuts the connective fibres nearer this pole than the other. In the second maturation also the two centrosomes are unequally developed. Likewise Vejdovsky and Mrazek ('98) find in the late anaphase of the first cleavage of *Rhynchelmis* that the centrosome (*Tochterperiplast*) and sphere (*Mutterperiplast*) at one pole are much larger than those at the other, corresponding to the fact that the first cleavage is very unequal in this animal. A similar disparity in the size of the two centrosomes and asters has been observed by Wilson ('94) in *Nereis*, Kostanecki and Wierzejski ('96) in the first maturation of *Physa*, Lillie ('99) in the first cleavage of *Unio;* but in all these cases except possibly that of *Actinosphærium* the disparity does not appear until after the spindle has taken an eccentric position in the cell.

In all the divisions of eggs and blastomeres, among the gasteropods which I have studied, not only the centrosome but every portion of the mitotic figure divides with exact equality, however unequal the approaching cell division may be. The chromosomes not only divide equally, but each half is a mirrored image of the other in shape as well as size. If one may judge by the form of daughter chromosomes, the division is qualitatively as well as quantitatively equal. The centrosomes and asters at the two poles of the spindle are also exactly equal during the earlier stages of division and until the eccentricity of the mitotic figure becomes so great as to limit the size of the peripheral aster (*cf.* Conklin '94, Wilson '96). Then the centrosome, sphere and aster at the peripheral pole grow smaller than those at the opposite pole and even before the daughter cells are separated they are proportional in size to the volume of cytoplasm in the two cells, figs. 73, 90, text figs. VII–XI. Even after the separation of the daughter cells the nuclei may be entirely equal, but ultimately they also become proportional in size to the volume of cytoplasm in which they lie, and in the next division of these nuclei the chromosomes are proportional in size to the nuclei from which they come.

One may therefore construct a table of the relative sizes of various cell constituents, all of which are ultimately reducible to the volume of cytoplasm within the cell.

Volume of	the	Centrosome	is proportional	to	that	of the Sphere
"	"	Sphere	"	"	"	Aster
"	"	Aster	"	"	"	Cytoplasm
"	"	Chromosome	"	"	"	Nucleus
"	"	Nucleolus	"	"	"	Nucleus
"	"	Spindle	"	"	"	Nucleus
"	"	Nucleus	"	"	"	Cytoplasm

The size of all these cell constituents, therefore, is dependent upon the volume of the cytoplasm, and there is the best of evidence that the eccentricity of the

mitotic figure, which precedes unequal cleavage, is itself a result, rather than a cause, of cytoplasmic structure and activity. This is shown especially well in the formation of the polar bodies in *Crepidula*. The two centrosomes and asters are here absolutely equal until one pole of the spindle comes into contact with the egg membrane. Then a lobe of cytoplasm is formed over this pole and the peripheral movement of the spindle continues until the centrosome and chromosomes at the peripheral pole are thrust clear through this lobe into contact with its distal cell wall. Then the whole spindle becomes shorter and stouter, the connecting fibres being curved and bent, showing that the shortening of the spindle is due to some force entirely outside of the spindle which is propelling it against the cell wall. Such a case shows in the clearest possible manner that the eccentric position of the spindle is the result of cytoplasmic activities.

In the cleavage the same fact is apparent in every unequal division. Such divisions are preceded by an eccentricity of the mitotic figure, but this in turn is caused by active movements of the cytoplasm. In fact one may frequently be able to determine that a given cleavage will be unequal long before the spindle is formed, by the position of the nucleus during the rest. In the first quartette cells of *Crepidula* the nuclei lie in the inner angles of the cells during the rest, figs. 91, 93, and when the next spindle is formed and the nuclear membrane dissolved the entire mitotic figure moves away from the animal pole until the peripheral pole of the spindle come into contact with the cell membrane at the outer side of the cell and here the small peripheral trochoblast is separated from the large apical cephaloblast, figs. 93–96. In short the nucleus in the resting period preceding division lay entirely within the area of the future larger cell. The same phenomenon is shown in the division of the cephaloblasts (*cf.* figs. 96, 97).

The conclusion that the eccentricity of the spindle is caused by the activity of the cytoplasm is supported by the observations of other authors, particularly by Lillie's ('99, '01) work on *Unio*. The first cleavage in this animal is quite unequal yet "the spindle forms in the center of the egg in the plane already indicated by the elongation of the sphere substance. . . The entire spindle then moves directly along the prolongation of its axis, and thus parallel to the direction of elongation of the sphere substance, to one side of the egg, until the centrosome of one end comes almost into contact with the peripheral layer of protoplasm." Then the spindle again moves back toward the center of the egg, and then again toward the periphery until it finally comes to rest with its equator in the plane of the future cleavage. These movements of the spindle Lillie attributed to the orientation of the cytoplasm.

Somewhat similar oscillatory movements of the spindle have been observed by Ziegler ('95) in the living eggs of nematodes, though he did not connect them directly with equality or inequality of division. In my first paper on this subject ('94) I also called attention to the oscillatory movements of nuclei and cytoplasm during cleavage and pointed out the relation of such movements to the direction and equality of division.

In conclusion, it is obvious that in *Crepidula* and *Unio* the place of cell divi-

sion is prearranged in the cytoplasm and that in normal cell division the mitotic figure is oriented by stresses and movements within the cytoplasm which bring the spindle to rest with its equator in the plane of cleavage. The equality or inequality of cell division is therefore a function of the cytoplasm.

(*d*). *Quality of Daughter Cells.*—Finally we consider homogenous and heterogenous divisions or the differential distribution of different substances to daughter cells. In the case of both nucleus and centrosome there is every evidence that the most exact halving and distribution of their substance, not only quantitatively but qualitatively as well, occurs at every division. So far as my observations on the gasteropods go there is absolutely no evidence that centrosomes or chromosomes undergo the slightest qualitative changes as development advances.

With the cytoplasm, however, the case is quite different; there is here not only differential distribution of yolk but also of sphere substance and of different kinds of cytoplasm (*viz.* granular or hyaline.) Thus the yolk is entirely contained in the macromeres while the micromeres are wholly free from it. The sphere substance too, after the first two cleavages, is differentially distributed at every division, always passing into that daughter cell which lies nearer the animal pole. Here it slowly disintegrates and disappears and in its place a clear hyaline kind of plasma is formed (see text figs. XIX–XXV). If the sphere substance, or the plasma into which it is transformed, maintains the same kind of polarity after its transformation that it had before, there would result an aggregation of this substance or plasma in the cells lying near the animal pole. The result of this differential distribution of the sphere substance may be summarized as follows:—The first quartette contains two and one-half generations of sphere substance, *i. e.*, all the sphere substance of the first and second cleavages and one-half that of the third; the second, third and fourth quartettes each contain one generation of sphere substance, *i. e.*, one-half that of the division by which they were formed and one-half that of the preceding division. The macromeres never contain more than one-half generation of sphere substance.

Finally, in the subdivisions of the quartettes, the cells lying nearest the animal pole receive most of the sphere substance or of the plasma to which it gives rise. Since the sphere substance varies in quantity in different cells, being always proportional to the size of the cell, the distribution of the substance by generations does not give any idea of its quantitative distribution. However, the first quartette not only receives a larger number of generations of the sphere substance than any other but also a larger quantity of this substance. Associated with this may be the fact that the cytoplasm of the first quartette is always clearer and less granular than that of the second and third.

The sphere substance is formed of hyaloplasm from the cell body and achromatin from the nucleus and the differential distribution of this substance may be an important factor of differentiation. If the nucleus controls the cell as DeVries, Weismann and Roux maintain,[1] we have in this differential distribution of the spheres a possible mechanism for such control, as well as for differentitation. However, the

[1] See p. 52.

only difference in cells which I can positively associate with this differential distribution of the spheres is the more or less hyaline character of the cytoplasm.

This differential distribution of the spheres, like the distribution of the cytoplasm and yolk, is the result of the polarity of the cell contents; the mechanism of this distribution is found in the cell movements during every telophase.

It follows from these conclusions on differential cell division that the various forms of cleavage such as radial, spiral, bilateral, equal, unequal, homogeneous, heterogeneous, etc., are expressions of the activity and structure of the cytoplasm rather than of the nucleus or centrosome, and since the cytoplasm is almost exclusively derived from the egg cell while very little of it comes from the sperm we should expect that the early cleavages would be little influenced by the latter. This is just what Boveri ('92) found to be the case in eggs of *Sphærechinus* which were fertilized by *Echinus* sperm. From this cross a larval form developed which was intermediate in character between the two genera, but the cleavage was purely maternal in character, thus indicating that it was not influenced by the sperm. Driesch ('98), also, in many crosses between different species of echinids has shown that the rythm of division, the vacuolization of cells of the blastula, the configuration of the larval stages, the color, manner of swimming and the number of mesenchyme cells of the larvæ depend upon the egg cell and not upon the sperm, and therefore, in all probabilities, upon the cytoplasm and not upon the nucleus or centrosome.

On first thought such conclusions seem to be at variance with the usually accepted view that the nucleus is the bearer of inheritance and that it, together with the centrosome, are the prime movers in all formative processes. They do not, however, do more than show that in the early development inherited characteristics, like material substance, are chiefly derived from the mother. But although differentiations and inherited characteristics first appear in the cytoplasm there is good reason to believe that the structure of the latter is influenced by the nucleus through the large amount of nuclear material which escapes into the cytoplasm at every mitosis. Certainly many features of later development are derived from the father and the conclusions as to the part which the nucleus has in hereditary transmission, founded as they are upon the remarkable apparatus for such transmission afforded by the nuclei, cannot be lightly cast aside.

I have attempted to show in what manner the cytoplasm is responsible for some of the early differentiations of development; how many important features of polarity and differential cell division are caused by movements of the cytoplasm; how these movements are perhaps caused by chemotropic attractions between unlike substances; but if we go farther and inquire what directs and co-ordinates these cytoplasmic movements we cannot at present find any satisfactory answer. It may of course be said that this is due to the "structure of the cytoplasm", but this is no more than a convenient phrase to include a whole series of more or less unknown phenomena which must still be analyzed.

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EXPLANATION OF FIGURES.

All figures are camera drawings of eggs of *Crepidula plana* and, unless otherwise specified, were drawn at the stage level under Zeiss apochromatic Obj. 1.5 mm., Occ. 4. The colors used are more or less conventional, no attempt having been made to reproduce the exact coloring of the preparations; in all cases yolk is represented in yellow and cytoplasm in neutral tint.

REFERENCE LETTERS.

Am^{I}.—Amphiaster (Netrum) of the First Maturation.
Am^{II}.— " " " Second "
Am^{1}. Am^{4}.—Amphiasters of successive Cleavages.
As.—Accessory Aster.
C^{I}.—Centrosomes of the First Maturation.
C^{II}.— " " Second "
C^{1}. C^{4}.—Centrosomes of successive Cleavages.
Ch.—Chromosomes.
Ch. V.—Chromosomal Vesicle.
FS.—Fused Spheres.
H.—Head of Sperm.
M.—Middle piece of Sperm.
MG.—Middle piece Granules.
N.—Nucleus.
Nl.—Nucleolus.
Nl^{2}.—Inner Nucleolus.
S.—Sphere.
S^{1}. S^{4}.—Spheres of successive Cleavages.
T.—Tail of Sperm.
Z.—Zwischenkörper.
1a-1d.—Micromeres of the First Quartette.
2a-2d.— " " Second "
A, B, C, D.—Macromeres.

Plate I.

First Maturation Division of Crepidula plana.

Fig. 1.—Ovarian egg showing germinal vesicle and spot, the latter composed of two parts.

Fig. 2.—Unfertilized egg from the uterus; optical section showing what appear to be two centrosomes lying next the nucleus. Obj. 3 mm., Occ. 4 (Zeiss Apochromat).

Figs. 3 and 4.—Fertilized eggs from the uterus showing indentation of the nuclear membrane, linin network and growth of chromosomes. Obj. $\frac{1}{16}$ (Leitz), Occ. 4.

Figs. 5 7.—Fertilized eggs just laid; formation of spindle and elimination of chromatin and nucleolus.

Fig. 8.—Prophase of first maturation; chromosomes of various shapes, centrosomes irregular in outline.

Fig. 8a.—Tangential section through centrosome and sphere of a stage similar to Fig. 8.

Figs. 9 and 10.—Early metaphase showing two characteristic positions of spindle. Obj. 3 mm., Occ. 4.

Fig. 11.—Same as preceding, showing cross-shaped chromosomes and irregular centrosomes with light center.

Fig. 12.—A characteristic metaphase; chromosomes accurately drawn both as to form and position; the chromosome Ch^2. is displaced out of spindle, probably by the knife. Centrosomes show clear center.

Fig. 12a.—Cross section through the equator of a spindle of the stage of preceeding. Processes of spindle substance radiate into the cytoplasm. Many chromosomes are surrounded by a linin sheath.

Fig. 13.—Early metakinesis; the daughter chromosomes are connected by linin threads which in some cases are moniliform. The centrosomes are hollow and the spheres more regular than in preceding stages.

Fig. 14.—Anaphase; interfilar substance of spindle between chromosomes and spheres; chromosomes with faintly staining centers; centrosomes contain a hollow central corpuscle; peripherel centrosome and sphere flattened against the egg membrane.

Fig. 15.—The chromosomes lie at the borders of the spheres; centrosomes and central corpuscles elliptical; protrusion of outer pole of spindle.

Fig. 16a.—Connective fibres granular; central corpuscle transformed into amphiaster (netrum) of second maturation division; outer pole of spindle not protruding.

Fig. 16.—Slightly later stage than preceeding; chromosomes pass into spheres; centrosomes and inclosed amphiasters (netra) at both poles; protrusion of first polar body.

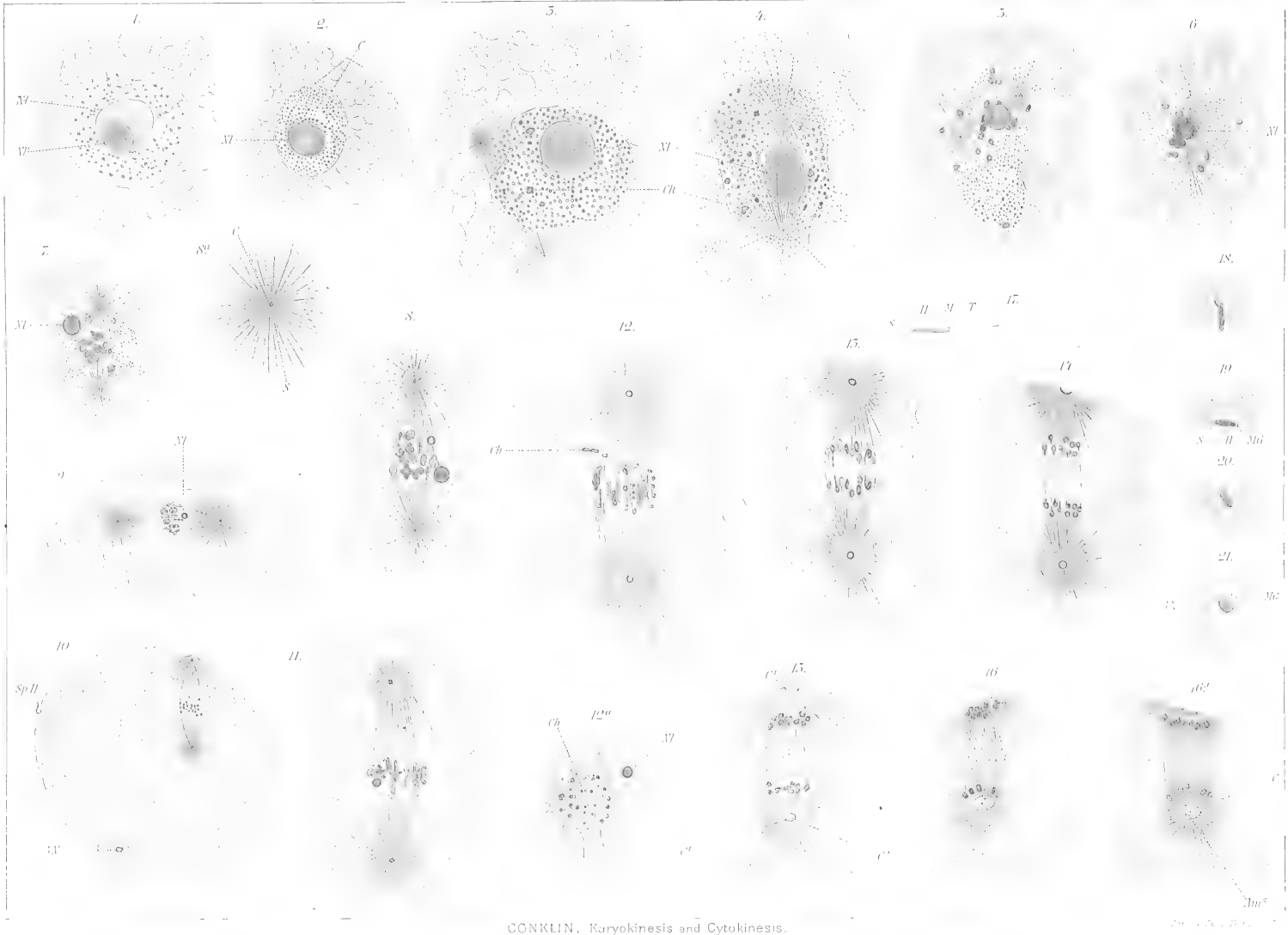
Fig. 17.—Mature spermatozoon of *C. plana*.

Fig. 18.—Sperm head immediately after entering the egg from a preparation of the same stage as Fig. 3.

Fig. 19.—Rotation of sperm head; appearance of middle piece granules; egg in stage of Fig. 4.

Fig. 20.—Shortening of sperm head; egg in stage of Figs. 5–7.

Fig. 21.—Formation of sperm nucleus; egg in stage of Figs. 10–14.



CONKLIN. Karyokinesis and Cytokinesis.

Plate II.

Second Maturation and Fecundation of C. plana.

Fig. 22.—Separation of first polar body ; amphiaster (netrum) of second maturation still within the old centrosome, amphiaster also in the first polar body.

Fig. 23.—Similar to preceding ; formation of ring-shaped mid-body (*Zwischenkörper*).

Figs. 24 and 25.—Growth of amphiaster within egg centrosome.

Fig. 26.—Outlines of the old centrosome have disappeared ; amphiaster lies free in the cytoplasm surrounded by chromosomes ; the new astral rays are independent of the old, which are centered on the amphiaster as a whole.

Figs. 27 and 28.—Two different positions of the second maturation spindle.

Fig. 28a.—Early stage in division of the first polar body. Obj. $\frac{1}{16}$ (Leitz), Occ. 4.

Fig. 29.—Spindle much stouter than in previous stages ; outer pole of spindle lies immediately under the mid-body (*Zwischenkörper*).

Fig. 30.—Metaphase of second maturation; cross-shaped chromosomes in maturation spindle and in first polar body.

Fig. 31.—Tetrad-like chromosomes in the second maturation spindle ; separation of the halves of these chromosomes into dumb-bell shaped bodies.

Fig. 32.—Anaphase of second maturation ; chromosomes dumb-bell and rod shaped ; centrosomes hollow ; first polar body divided.

Fig. 33.—Stage a little later than preceding ; chromosomes rounded, connective fibres granular ; central area of centrosome granular.

Fig. 34.—Separation of second polar body and division of first ; granular centrosome and sphere left in egg ; chromosomes vesicular ; mid-body well marked.

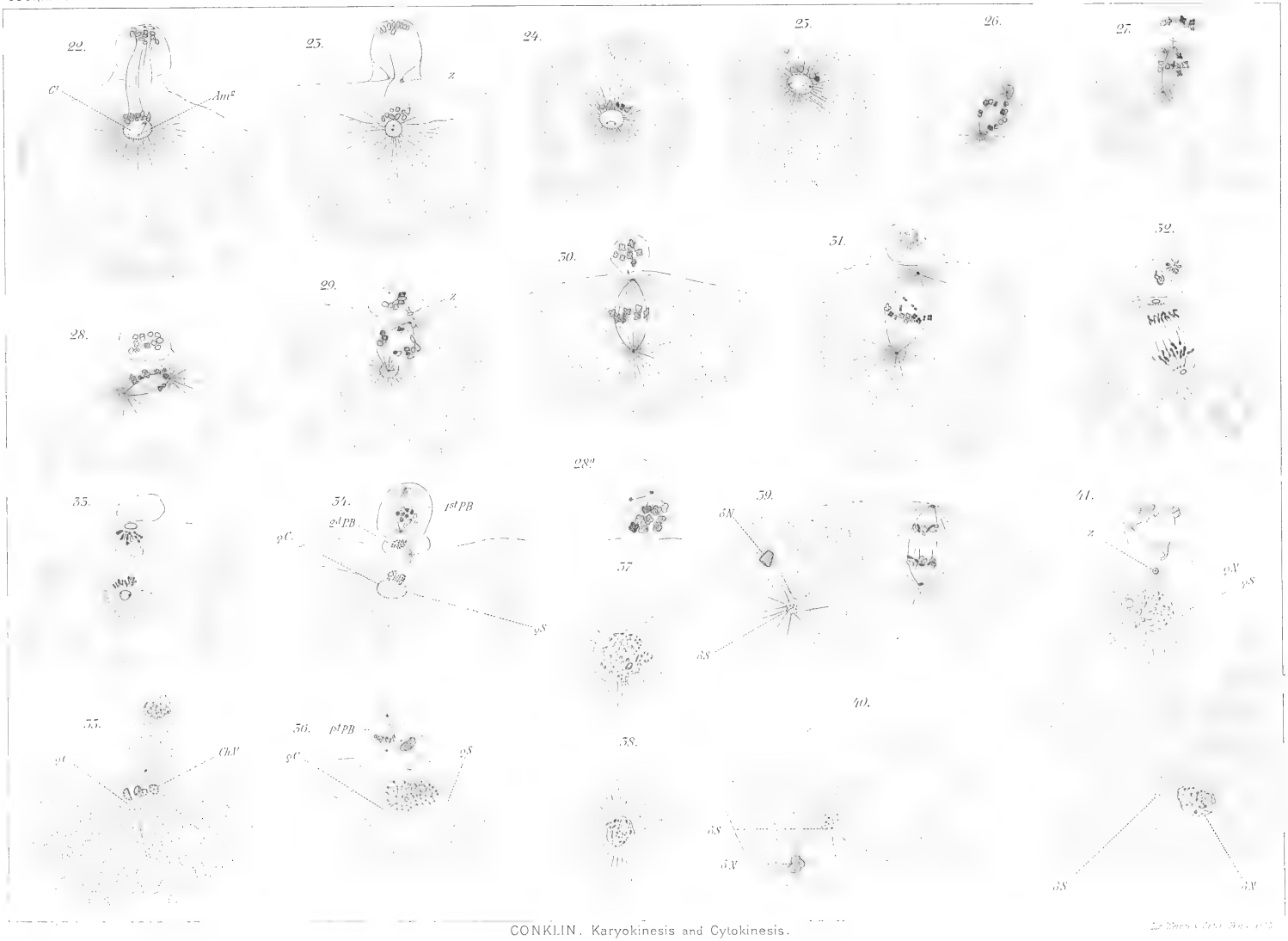
Fig. 35.—Similar to preceding ; chromosomal vesicles are surrounded by the sphere.

Fig. 36.—Reticular egg nucleus present and surrounded by the sphere ; centrosome a faint granular mass.

Figs. 37 and 38.—Polar views of egg nucleus surrounded by sphere.

Figs. 39 and 40.—Anaphase of second maturation ; first appearance of sperm aster around granules of middle piece.

Fig. 41.—Approach of sperm nucleus and sphere to those of egg.



CONKLIN. Karyokinesis and Cytokinesis.

Plate III.

Fecundation and First Cleavage of C. plana

Figs. 42 and 43.—Approach of sperm nucleus and sphere to those of egg; one accessory aster in each egg; one large accessory aster present. Obj. 3 mm., Occ. 4.

Fig. 44.—Union of egg sphere and sperm sphere; contact of germ nuclei. Obj. 3 mm., Occ. 4.

Fig. 45.—Germ nuclei in contact; spheres still distinct; yolk granules between the two spheres and nuclei.

Fig. 46.—Egg and sperm spheres fused and yolk granules inclosed. Obj. 3 mm., Occ. 4.

Fig. 47.—The fused spheres surround the germ nuclei; cleavage centrosomes appear at border of spheres. Obj. 3 mm., Occ. 4.

Fig. 48.—Sphere substance no longer visible; one centrosome and sphere in connection with each germ nucleus; same stage as Fig. 53. Obj. 3 mm., Occ. 4.

Fig. 49.—Germ nuclei surrounded by the fused spheres.

Figs. 50 and 51.—Similar to preceding; cleavage centrosomes appear at the border of the fused spheres; chromatin granules vesicular.

Fig. 52.—Cleavage centrosomes at the poles of the germ nuclei; no central spindle; chromatin granules vesicular. Obj. $\frac{1}{16}$ (Leitz), Occ. 4.

Fig. 53.—One centrosome and half spindle in connection with each germ nucleus; nuclear membrane indented opposite centrosomes; chromosomes forming out of granules, other granules dissolving in nuclear sap.

Fig. 54.—First appearance of central spindle between the two cleavage centrosomes; chromosomes aggregating in the spindle; other granules dissolving; spheres increasing in size and substance radiating from them through the cytoplasm.

Fig. 55.—Prophase of first cleavage; oxychromatin granules arranged along the spindle fibres. Obj. $\frac{1}{16}$ (Leitz), Occ. 4.

Fig. 56.—Metaphase of first cleavage; "heterotypic" division of the chromosomes.

Fig. 57.—Similar to preceding; centrosomes slightly hollow.

Fig. 58.—Anaphase of first cleavage; centrosomes hollow; spheres alveolar.

Fig. 59.—Late anaphase; chromosomal vesicles closely appressed to sphere; centrosomes and spheres filled with a delicate reticulum.

Fig. 60.—Telophase of first cleavage; beginning of the bending of the spindle axis; nucleus in the left cell partly divided into two portions of which the upper half is from the egg nucleus and the lower from the sperm.

Fig. 61.—Resting stage at close of first cleavage; spindle axis bent on itself through nearly 180°; nuclei almost in contact with each other, centrosomes and spheres near surface and mid-body carried down to the middle of the egg. The mid-body is a hollow sphere, like a centrosome, surrounded by a dark area, like a sphere.

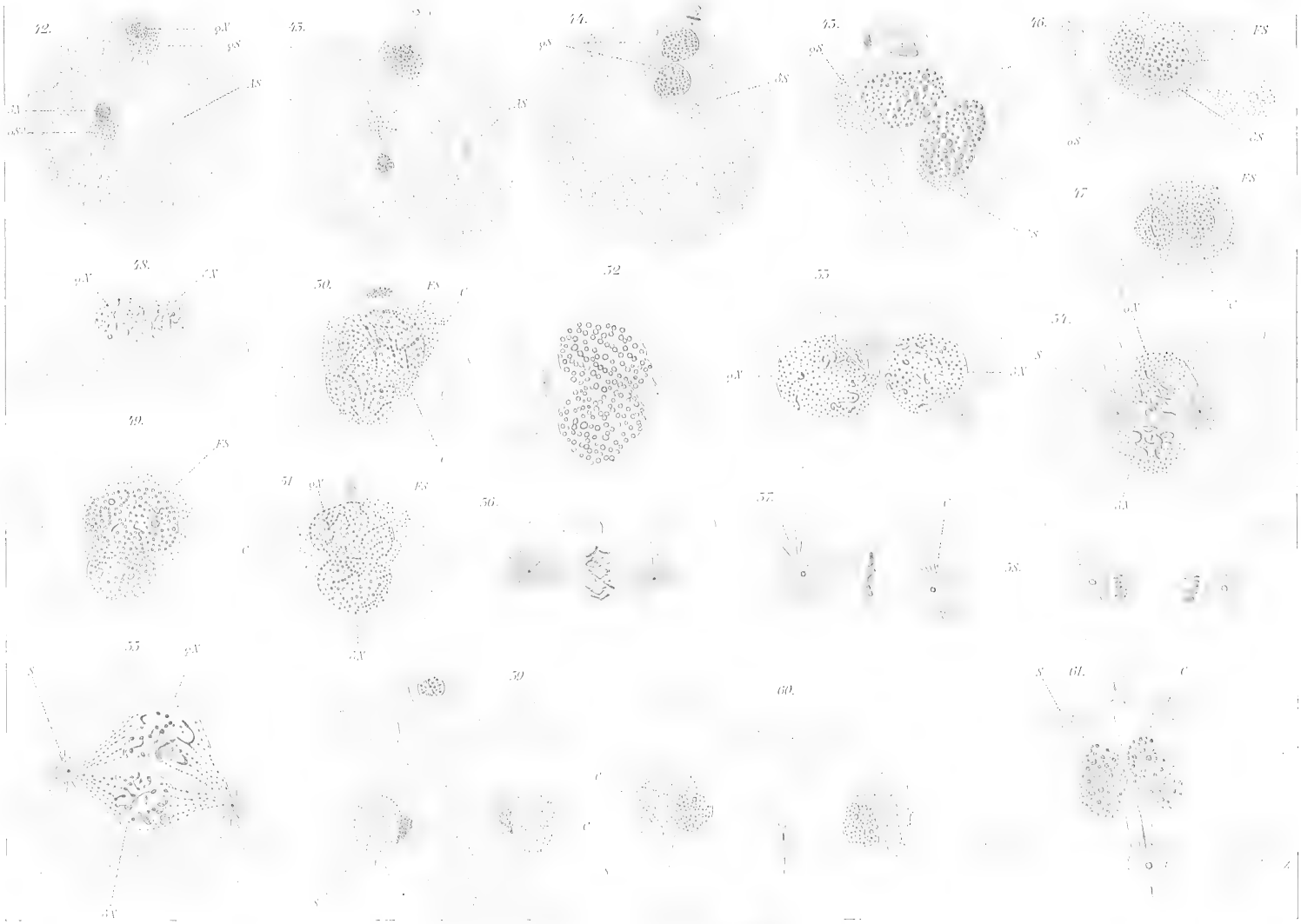

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Plate IV.

Second, Third and Fourth Cleavages of C. plana.

Fig. 62.—Similar to preceding ; nucleus in early prophase.

Fig. 63.—Section at right angles to preceding ; amphiaster for second cleavage present.

Fig. 64.—Prophase of second cleavage. (Omitted from plate for lack of space and put in text as fig. XVIII.)

Fig. 65.—Metaphase of second cleavage.

Fig. 66.—Anaphase of second cleavage ; chromosomes at the borders of the spheres ; latter alveolar.

Fig. 67.—Anaphase of second cleavage ; chromosomal vesicles apparently absorbing substance from spheres.

Fig. 68.—Telophase of second cleavage (horizontal section) ; centrosomes faintly granular.

Fig. 69.—Rest stage at close of second cleavage ; centrosomes densely chromatic.

Fig. 70.—Amphiasters for third cleavage moving out of spheres and old centrosomes ; nuclei show polar differentiation of the chromatin.

Fig. 71.—Prophase of third cleavage ; nuclei indented ; sphere remnants at apical pole.

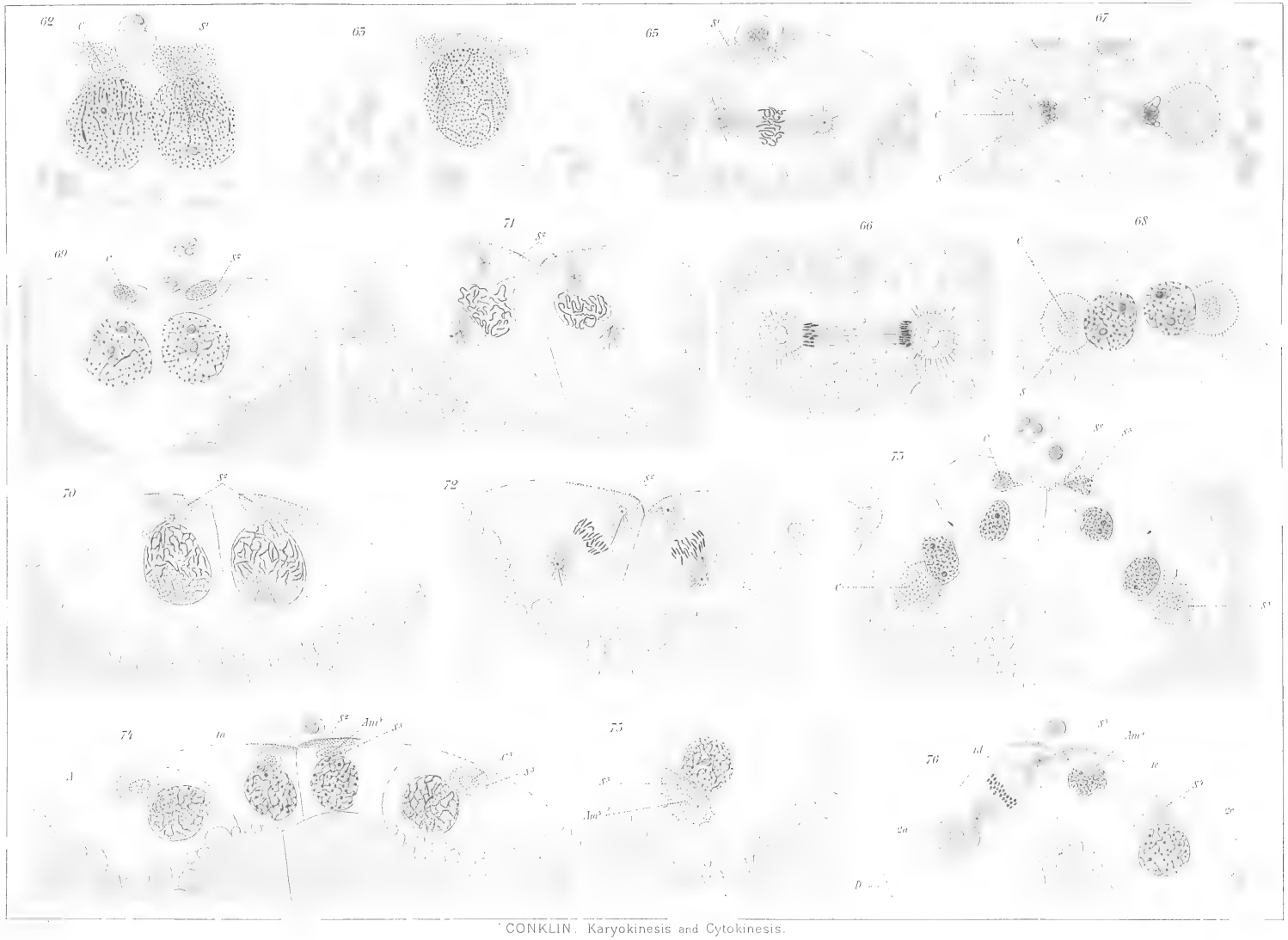
Fig. 72.—Metaphase of third cleavage.

Fig. 73.—Telophase of third cleavage.

Fig. 74.—Resting stage after third cleavage. Centrosomes are reticular spindles.

Fig. 75.—Same stage as preceding ; section through one macromore showing the centrosome, which becomes the amphiaster of the fourth cleavage, as a reticular spindle.

Fig. 76.—Subdivision of the first quartette ; spindle in metaphase in one cell ; amphiaster just escaped from the sphere in the other ; nucleus and spheres of the second quartette cells shown.



CONKLIN. Karyokinesis and Cytokinesis.

Plate V.

Entire Eggs of C. plana; one to four cells.

All the figures on Plates V and VI were drawn at the stage level under Zeiss Apochromat Obj. 3 mm., Occ. 4.

Fig. 77.—Entire egg showing in optical action sperm nucleus and sphere approaching egg nucleus, also accessory asters and yolk lobe. There is a single nucleolus in each germ nucleus.

Fig. 78.—Contact of germ nuclei and fusion of spheres.

Fig. 79.—Anaphase of first cleavage viewed from animal pole. The fused spheres lie above the spindle and the polar bodies above the spheres.

Fig. 80.—Early telophase of first cleavage, showing dual nuclei, equatorial constriction of egg, yolk lobe and early bending of spindle axis.

Fig. 81.—Late telophase of first cleavage, showing dual nuclei and extensive bending of spindle axis.

Fig. 82.—Two cell stage from animal pole; spheres and centrosomes lie above nuclei. In right blastomere centrosome has given rise to new initial spindle.

Fig. 83.—Early prophase of second cleavage; sphere remnants lie close to polar bodies; initial spindle at outer and upper side of nucleus in position of groove between the egg and sperm halves.

Fig. 84.—Metaphase of second cleavage; sphere remnants of first cleavage beneath polar bodies; spindles at outer sides of old nuclei; outlines of the latter still preserved.

Fig. 85.—Anaphase of second cleavage; equatorial constriction beginning as a depression beneath polar bodies; the margins of this depression are raised into many pointed processes.

Fig. 86.—Late telophase of second cleavage; bending of spindle axis shown by relative positions of mid-bodies, nuclei and centrosomes; dual nuclei.

Fig. 87.—Resting 4-cell stage; spheres in the apical angles of the cells; centrosomes not visible.

Fig. 88.—Prophase of the third cleavage; sphere remnants as in preceding figure; the spindle axes lie in various directions in the different cells; the nuclear outlines still indicated, also the escape of nuclear sap into the spheres.

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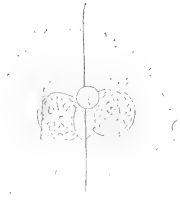

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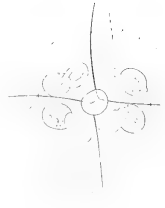

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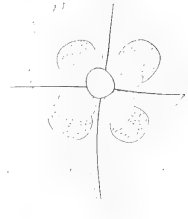

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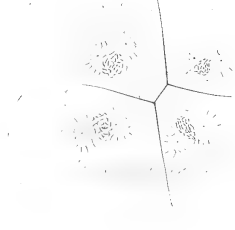

Plate VI.

Entire Eggs of C. plana; four to forty-five cells.

(The figures of this plate are oriented as in the preceding one, the first cleavage furrow running from top to bottom, the second from left to right; the left lower quadrant is A, the left upper B, the right upper C, and the right lower D.)

Fig. 89.—Metaphase of third cleavage; spindles in definitive positions, second cleavage sphere remnants still preserved at apical angles of cells.

Fig. 90.—Anaphase and telophase of third cleavage; rotation of cell contents and bending of spindle axes beginning; second cleavage sphere remnants still preserved; dual nuclei.

Fig. 91.—Resting 8-cell stage; rotation of cell contents and bending of spindle axes indicated by relative positions of yolk, cytoplasm, mid-bodies, nuclei and centrosomes; the latter are chromatic and elliptical or spindle-shaped.

Fig. 92.—Fourth cleavage and formation of second quartette; third cleavage sphere remnants at extreme left of each macromere, where they are spread into a broad ring by the upper pole of the spindle; centrosomes in micromeres spindle-shaped.

Fig. 93.—Telophase of fourth cleavage of macromeres; prophase of first division of first quartette; the mitotic figure in one of these micromeres (1a) has already begun to move from an apical to a peripheral position in the cell.

Fig. 94.—Slightly later stage than the preceding showing the extensive bending of the spindle axes in the second quartette cells. Three of the first quartette cells are in the metaphase, their spindles being in their definitive positions, the nucleus of the fourth (1d) is still in the apical angle of the cell; sphere remnants still lie in apical angles.

Fig. 95.—Later stage than preceding showing still greater bending of the spindle axes of the second quartette cells. In the first quartette three cells are in the anaphase, one in the metaphase; third cleavage sphere remnants still preserved.

Fig. 96.—Formation of third quartette; subdivision of second quartette, the sphere remnants in these cells going into one daughter cell only (the right one) and not into the other; telophase in division of first quartette, the nuclei showing dual character.

Fig. 97.—Second division of first quartette; the division in three of these cells (1a, 1b, 1c) is unequal, in the fourth (1d) it is nearly equal and is always later than in the others. The extensive rotations in the daughter cells of the second quartette is indicated by the bending of the spindle axes. The mesentoblast (4d) already formed.

Fig. 98.—Resting 29-cell stage; the extensive bending of the spindle axes in the apical cells is shown by the positions of the spheres, in the basal cells of the cross this bending is very slight. A few dual nuclei are shown.

Fig. 99.—Forty-one cells; mesentoblast (4d) dividing; the second subdivision of the second quartette cells has occurred, the right product in each quadrant giving rise to the *tip cell* of the cross; the third quartette has also divided, the direction of the division being læotropic in quadrants A, B, C and slightly dexiotropic in quadrant D.

Fig. 100.—Forty-five cells; mesentoblast (4d) divided into right and left products (ME^l, ME^r); basal cells divided in three arms of the cross by a reversed cleavage; the basal cell in the fourth arm (posterior one) still undivided; dual nuclei are shown in the tip cells.

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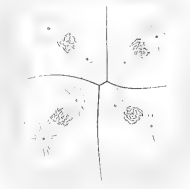

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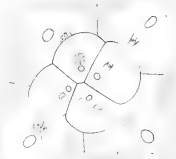

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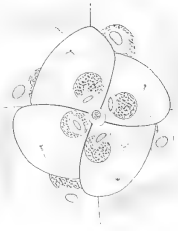

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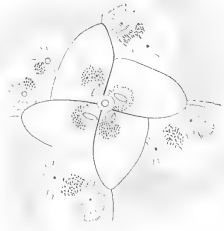

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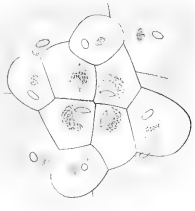

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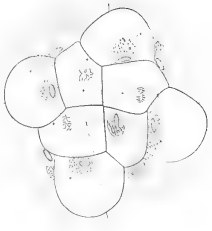

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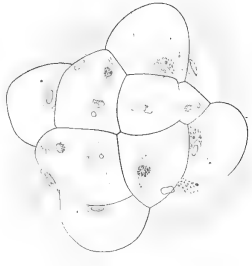

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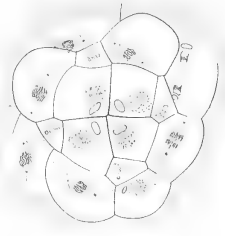

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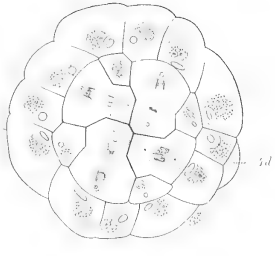

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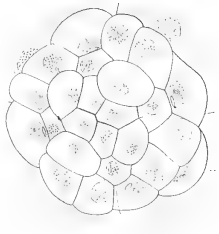

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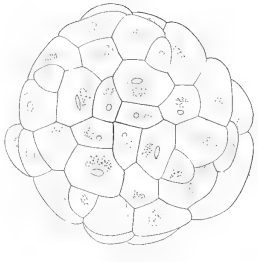

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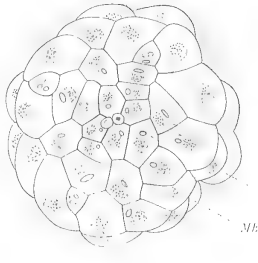

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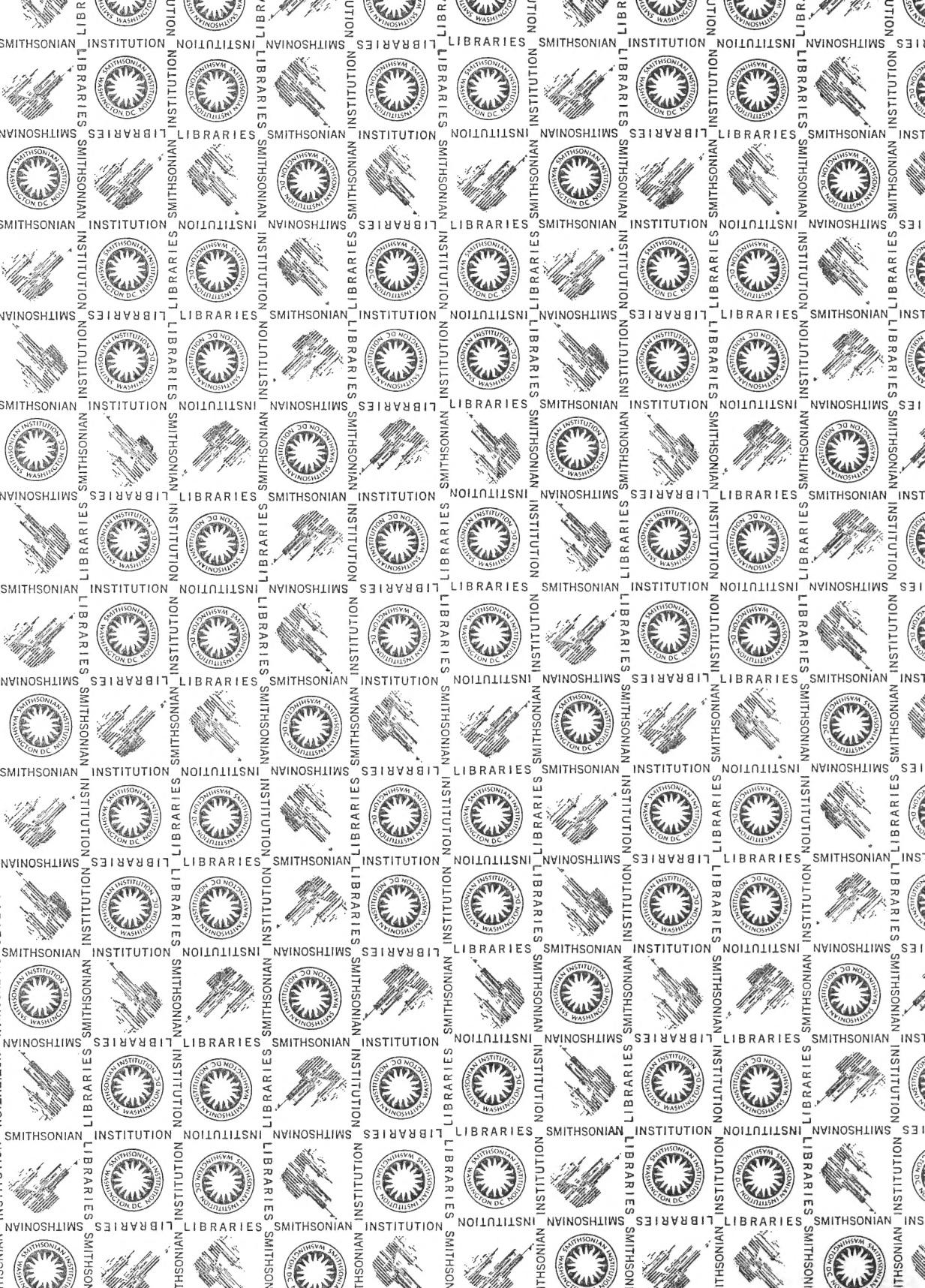
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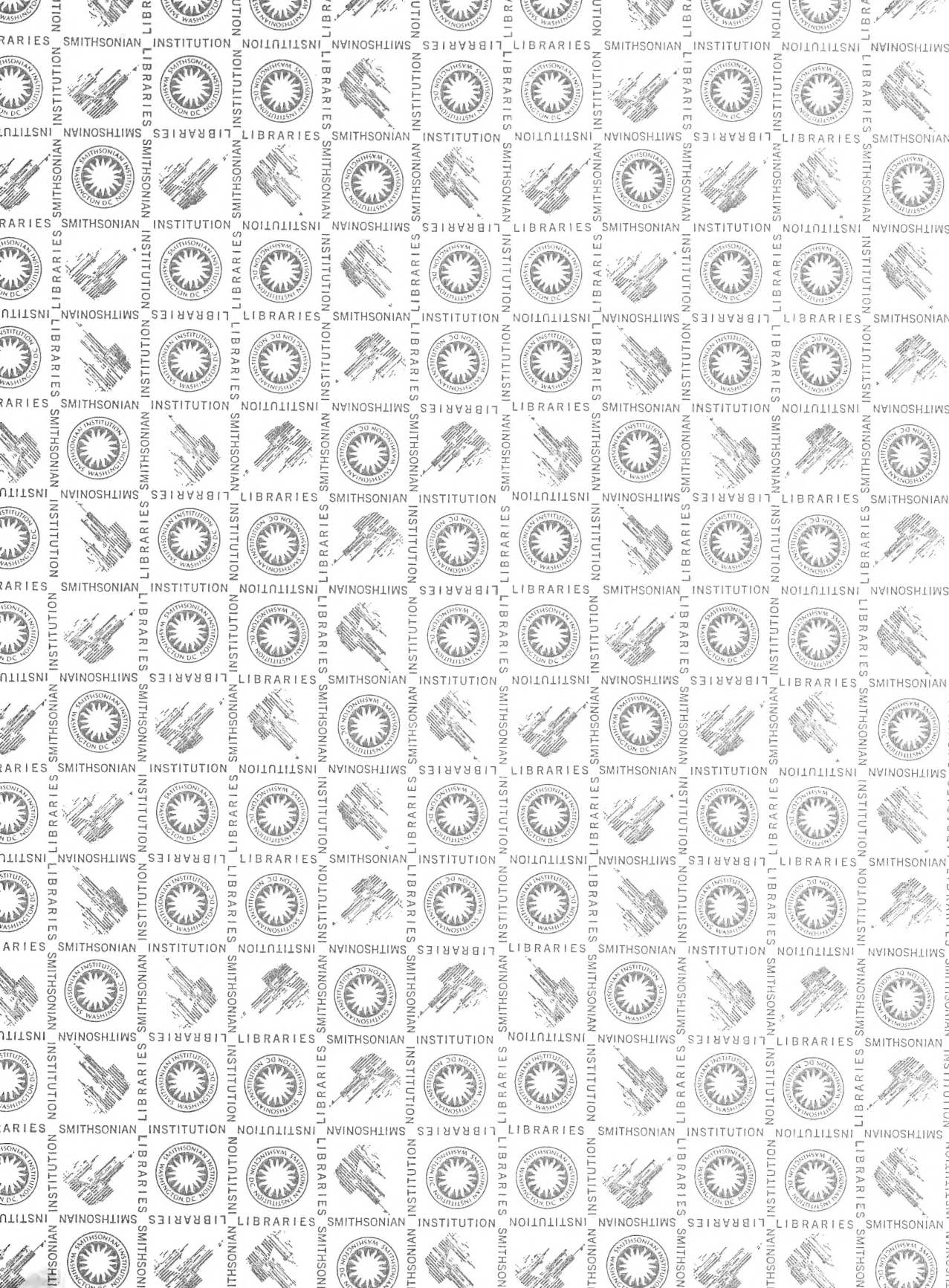
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